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Aim: To develop optical techniques for diagnosis and treatment of breast cancer

We developed the hardware for collection and analysis of white light reflected from tissue (elastic scattering spectroscopy, ESS) as a diagnostic technique. Paired optical and conventional histologic measurements were obtained from 1250 sites in breast tissue and axillary nodes. Using artificial intelligence techniques (neural networks, hierarchical cluster analysis) coupled with innovative spectral processing, we looked for spectral features to identify cancer by model based analysis (MBA). We achieved a sensitivity and specificity for detecting cancer in breast tissue of 94% and 92% respectively (84% and 87% in excised axillary nodes). ESS has the potential for instant, low cost detection of cancer without removing tissue from the patient in many tissues, justifying further study.

Therapy aimed for complete ablation of small cancers using percutaneous Interstitial Laser Photocoagulation (ILP). Safety studies treating fibroadenomas confirmed that laser necrosed tissue is resorbed and healing is without a scar or residual fibrous lump (ILP is now used routinely for fibroadenomas). Studies on cancers were limited by recruitment problems, but ILP could ablate small cancers and contrast enhanced MR could detect untreated areas of cancer as small as 2mm. Further studies should be undertaken.

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Final Report 1st September 1998 – 31st August 2002

Introduction:

The research conducted under the grant over the past four years falls broadly into two main areas:

1) *Diagnosis of malignant breast tissue, assessment of excision margins and detection of sentinel node metastases by optical biopsy*: This involves placing a probe containing two optic fibers into a breast lump, onto a suspicious area of tissue during surgery or onto an excised tissue specimen such as a lymph node. One fiber is attached to a Xenon flashlamp that sends short pulses of white light into the area of tissue to be interrogated. A second optic fiber collects light that is scattered back, which is then analyzed spectroscopically. A conventional histological specimen (a biopsy) is taken from precisely the same point, and when this has been analyzed the two sets of data are correlated. Algorithms for spectral analysis can subsequently be developed once sufficient data sets have been collected. In this way it is anticipated that we can train the optical system to give an accurate diagnosis of any breast lump, or predict the presence of malignant tissue in surgical excision margins or excised lymph nodes.

2) *Treatment of cancers and benign fibroadenomas of the breast by Interstitial Laser Photocoagulation*: The tumors to be treated are first identified by either ultrasound scanning (USS) or Magnetic Resonance Imaging (MRI). One or more small-bore cannulae are inserted into the middle of the lesion through which optic fibers are passed. These in turn are attached to a semi-conductor diode laser. The laser is activated for between 10 and 20 minutes at each fiber site. The laser causes heating of the tumor tissues and subsequent thermal necrosis. The body reabsorbs the dead tissue over a period of several months. For the fibroadenomas, follow up is with serial USS. Patients with cancer have laser treatment before routine surgery. They all have MR scans pre- and post laser treatment and just before surgery. These are subsequently compared to the histological specimen. We aimed to show that fibroadenomas and small cancers can be successfully treated with laser therapy and to determine if MR imaging can detect any malignant tissue remaining after laser treatment.

BODY

In the 4 year period covered by this report there have been a number of changes in personnel, including a relocation of the group representing one of the subcontractors and the discontinuation of the subcontract with a second. Our principle collaborator at Los Alamos, Dr Irving Bigio, moved to Boston University as Prof. of Biomedical Engineering during the third year of the project, although we continued our collaboration with him on the same terms as when he was at Los Alamos. The team led by Dr Steven Harms at Little Rock, Arkansas, have been unable to undertake the work described in their agreement with the principal investigator in London, and so with regret, that sub-contract has been terminated. A revised statement of work was submitted to the sponsors in March 2001. This was approved and the project was extended for a year with no extra funding.

Developments in the analysis of data have led to a patent application in the field of spectral processing and for this development the U.S. Army Medical Research and Materiel Command has been informed separately. The senior breast surgeon involved at the inception of this program, Christobel Saunders, emigrated to Australia and was replaced by Mr Mo Keshtgar, who has been directing the clinical side of the work for the last 2 years. He now provides a routine laser service for fibroadenomas, based on results that emerged from this program. Throughout, the program, Margaret Hall-Craggs has been the interventional radiologist. Gavin Briggs, the research surgeon who had been working on the project from its inception finished his contract and left to take up a position as a trainee radiologist in Belfast, Northern Ireland. He has submitted his research thesis (MD) and is awaiting his oral examination. Another surgeon, Andrew Lee, joined the project in the third year and is currently writing up his research results.

Objective 1: Advance diagnostic techniques with Optical Biopsy.

At the start of this program, little more than the basic principle of the use of elastic scattering spectroscopy for tissue diagnosis was known. In the first two years we undertook some basic experiments to understand what volume of tissue the pulse of white light was interrogating. We have also performed experiments to determine the absorption coefficients of various commonly observed exogenous dyes which may be introduced into tissue and colored substances (endogenous chromophores) which are known to occur naturally in tissue. This has led to new ways of interpreting the spectra obtained from tissue to make the analysis simpler and as accurate as possible. We have been looking particularly at ways of eliminating spectral features due to absorption of the light by the chromophores, thereby maximizing the information available from the rest of the spectrum. These spectral processing techniques have led to a patent application and the possibility of commercializing the technology.

Experimental work:

Assessment of the volume of tissue studied

From theoretical considerations, the distance from the fiber tip from which light may contribute to the detected optical signal is about 1mm. Our experiments confirmed this result. Tissue up

to a distance of 1mm from the tip of the probe could influence the spectrum, but tissue further away could not. We concluded that with the geometry of the probe as it is constructed at present the volume of tissue interrogated is of the order of $2mm^3$. Thus conventional biopsy specimens needed to be taken within about 1mm of the site of the optical measurement for accurate correlation of the two.

Absorption by hemoglobin

Usually the strongest chromophore in normal breast tissue in the visible part of the spectrum is hemoglobin (in both oxygenated and deoxygenated forms). In purely adipose tissue or if a blue dye has been used intra-operatively to locate the sentinel node then other chromophores may dominate. In our spectra, it was noticed that the hemoglobin absorption curves from different tissue types showed some obvious similarities. This raised the possibility of measuring the relative hemoglobin absorption within each spectrum. Not just might this give an approximation of the saturation (percentage oxygenation) and total hemoglobin concentration within the tissue but also this measurement could be used to attempt to remove the hemoglobin absorption contribution from each spectrum. If this could be done, it might reveal more diagnostic information from the remaining, underlying, parts of the spectrum.

The first problem was that the absorption spectra of oxy- and de-oxy hemoglobin, although similar in overall form, have slight differences in the position, width and height of their absorption maxima. The relative amounts of oxy- and de-oxy hemoglobin and the overall amount of blood in the tissue, as well as any concentration of blood between the tip of the probe and the tissue, could vary over a large range. However, the separate oxy-Hb and deoxy-Hb peaks are sufficiently different to be recognizable so two experiments were devised to quantify the values for the absorption spectra to be used on the data we collected from patients.

1) Firstly a sample of venous blood (nominal Hb 12g/dL) was acquired from one of the researchers and a series of samples with different concentrations were obtained with saline using a double dilution method. The concentrations ranged over 4 orders of magnitude i.e. 1:0 to 1:32768; blood:saline and ESS spectra were obtained from each concentration. A linear relationship was observed over 2 orders of magnitude spanning 1:256 to 1:32768 which corresponds to the concentrations observed in breast tissue. Using this data we can now obtain a relative concentration of blood for any given spectra, which could then be used as an additional factor in the analysis. The same pattern was observed when the dilutant mixture contained a scatterer (Intralipid 10%).

2) To assess deoxy- and oxyhaemoglobin absorption we used a venous blood:saline concentration of 1:512 (inside the linear range) and gently bubbled pure oxygen through the solution for 5-10 minutes, until no further color change was observed in the solution. A spectrum was obtained which therefore only reflected absorption from oxy-hemoglobin. The pure oxy-Hb absorption spectrum could be obtained by examining the negative logarithm of the intensities after subtracting a reference reading. Next, we bubbled pure nitrogen through the same solution for a further 5-10 minutes and obtained a spectrum, which reflected absorption by pure deoxy-hemoglobin. Using these two as approximate end points, it was then possible to produce a composite absorption curve for any value of blood

oxygenation, and this data could then be used to normalize tissue spectra after calculation of a relative value for oxygen concentration.

These two experiments have allowed us to account for, and hence normalize, different levels of saturation and amounts of blood on or within the tissue that is being sampled. This concept has also made us wonder if this technique could be used as a way of monitoring the level of oxygen saturation of blood at a single point and therefore as a monitor of certain treatments such as photodynamic therapy (PDT). This awaits further exploration.

Similar experiments to determine the absorption spectra, in the particular backscattering geometry of the instrument, have been conducted for the blue dye used in sentinel node location and are planned to determine the exact absorption pattern of β-carotene. The experiments to determine the absorption spectrum of blue dye involved diluting the dye in sterile water to obtain a series of solutions with varying concentrations of dye. The dye solutions were injected into a homogeneous fatty tissue phantom, for which pork fat and lard were used and readings were taken from the regions thus injected and from the unadulterated phantom. Comparing the spectra taken at different concentrations with each other and the background spectrum of the fatty matrix, normalizing these absorption coefficients and taking a weighted average gave a good approximation for the absorption spectrum.

Fiber probe development:

All fiber probes used in these studies have been designed and fabricated by co-investigators at Los Alamos National Laboratory, and later at Boston University and supplied to the clinical site. The optical geometry of all the probes has been standardized so as to provide maximum consistency of measurement conditions for all studies. For all probe designs the illumination fiber (carrying light from the pulsed arc lamp to the tissue) has a core of 400 μm diameter and the signal (collection) fiber has a core diameter of 200 μm. The separation of the two fibers centers is fixed at 350 μm, which has previously been shown to maximize the spectral sensitivity to scattering from cellular nuclei. From Monte-Carlo modeling studies the predicted volume of tissue that is probed optically is approximately 500 μm long, 300 μm wide and 300 μm deep, although from our experimental studies, there is some effect from tissue up to about 1mm from the probe tip. Further work examining the effects of different probe geometries, including different fiber sizes and different source-collection separations, are being investigated by Prof. Bigio's group.

Two different types of probes were fabricated for the first series of clinical measurements: one type for interstitial measurements through a transdermal needle, and the other for direct superficial measurement during open surgery. For the interstitial application two variations of probe have been fabricated. One provides the smallest possible total probe diameter, for use through 18-gauge needles. This type has a polyamide outer protective sheath with an external diameter of approximately 900 microns. However, all transdermal optical measurements are correlated with a core biopsy from the same site, so a second transdermal probe was designed to fit the inner diameter of the guide needle part of the standard core-biopsy needle (MANAM™ automatic cutting needle). This probe has a surgical stainless steel sheath with a diameter of about 1.78mm, which permits easy insertion through the core-biopsy needle.

The other general type of probe was designed for easier manipulation by surgeons during breast surgery. It has a 5-mm diameter outer sheath of surgical stainless steel, and the fibers are fixed in the center, the remaining volume being filled with optically opaque epoxy. This probe design is easier to handle for surgeons wearing gloves, and helps to reduce the intensity of ambient light that reaches the collection fiber.

We have conducted studies of robustness of our probes against various types of sterilization. Ethylene oxide can be used with any of them, but that method is expensive and must be scheduled one or two days in advance of the expected use. The probes can also be disinfected with standard immersion fluids (such as glutaraldehyde), but this requires keeping the SMA connector ends of the probes out of the fluid because of possible corrosion of the polished metal mating parts. Moreover, many hospitals are reducing the use of biostatic fluids both because of potential harm to workers and environment, and because of some questions about reliability of sterilization. Ideally, we wish to be able to sterilize the probes by autoclave, which is reliable, safe and inexpensive. To that end we have changed some of the medical-grade materials used, especially the bonding epoxies, and have successfully tested the various components of the probes in several autoclave cycles, although we are still short of developing a fully autoclavable probe.

We have begun experimental testing of fiber probes using illumination fibers with larger numerical aperture than that of standard quartz fibers, as large as 0.40. We expect that this will permit tighter fiber bends without light loss, when compared with the standard fiber numerical aperture of 0.22. This would provide advantages for use in tight working environments, where the fibers may undergo severe bending between the probe and the spectrometer unit. We are also looking at developing smaller and more portable spectrometer units, which require less floor space in theatre, are easier to take from one location to another and are faster to set up, therefore allowing more data to be collected.

Patient enrolment, optical biopsy and histological sampling:

Clinical measurements were taken with both types of fiber developed at Los Alamos. The first studies were undertaken on excised human breast tissue within 1 hour of surgery as in this situation it is straightforward to be sure that optical measurements and tissue biopsies are taken from exactly the same site. These optical readings were obtained with the transmitting and detecting optical fibers held in the rigid 5 mm diameter probe. This was convenient for laboratory and open surgery studies but was too large for percutaneous measurements for which the smaller flexible probe was developed, small enough to pass through an 18-gauge biopsy needle. Following the initial studies on excised breast tissue, optical measurements have been obtained in three situations. All patients were enrolled into the study after the details had been explained to them and they had given full written consent.

To test the viability of taking ex-vivo measurements we conducted an experiment to determine how the optical properties of tissue change after removal from the body i.e. changes due to cooling, dehydration, loss of circulating blood and possible de-oxygenation. If we could show that the optical readings from breast tissue were essentially similar both *in-* and *ex-vivo* then it

would be possible to obtain readings from mastectomy specimens immediately after excision in the operating room. This would greatly increase the number of data sets we could obtain for training the system. One method of testing this was to mark a small area of breast tissue in a tumor bed following wide local excision of a breast cancer and obtain an optical reading *in-vivo*. This area would then be excised with a small cuff of surrounding tissue and serial measurements would be taken over time on the same point. Alternatively in order to define the point of observation uniquely and maintain the position of the probe a cannula from an appropriately gauged needle was sewn into position within the incompletely excised specimen and a spectrum was obtained. After the excision was complete the probe was re-inserted into the cannula and a timed series of spectra were obtained over a period of up to two hours. Although variants of this procedure have been performed on fifteen patients to date the numbers are not large enough to draw absolute conclusions although they strongly suggest that spectra obtained within a period of thirty minutes following excision contain very similar information to those taken in-vivo. Over time, a gradual shift in the oxygen absorption curves was seen, although the overall shape of the spectra did not change remarkably. Analysis is ongoing to determine if the small change is significant before embarking on obtaining further *ex-vivo* measurements. In the meantime we have concentrated on obtaining as many *in-vivo* measurements as possible by the methods outlined below with any ex-vivo measurements clearly labeled with the time elapsed after excision.

As each patient is enrolled in the study it is necessary to record certain relevant information in order to compare the measurements taken. This includes aspects of their medical history, including method and result of diagnosis, endocrine status, menopausal status and details of the procedure being performed. In order to take the appropriate information from each patient we have developed a pro-forma, which can be completed from details in the patients' notes and then later added to an electronic database.

a) Percutaneous measurement:

These were done on patients undergoing surgery for either benign or malignant conditions of the breast. Once the patient was anesthetized, a core-cut (14 French gauge) biopsy needle was inserted into the tumor through the skin (in the case of cancers or suspected cancers, the needle was inserted through skin that would be removed as part of the surgical procedure). When it was in the correct position, the inner needle was removed and the thin optical probe inserted for measurements to be taken. Once a satisfactory spectrum had been obtained, the core-cut needle was reassembled taking great care not to move the tip of the needle relative to the tumor. A standard tissue biopsy could then be obtained which contained the area of tissue that had been interrogated optically. After this, the scheduled surgery was performed.

To correlate the histology unambiguously with the spectra acquired it was necessary to indicate to the pathologists the portion of the acquired core that should be examined. The core-cut device takes a sample of tissue approximately 1.5mm in diameter and approximately 1cm long. Because the optical probe would only have measured the nearest regions of the core we adopted a system of marking the opposite end of the core, furthest away from the probe with India ink and then requesting that the histologist report the non-inked end of the sample separately. It was also agreed that the histologist would report the pathology of the sample according to

certain specific criteria, which are detailed in a core cut biopsy reporting pro-forma. An example form was attached to a previous report which illustrated the categories of normal tissue and benign or malignant breast pathology that the pathologist will classify the relevant end of the core into. Note that the statement requires the pathology within the first 2mm to be reported in accordance with the experimental work detailed above and in our previous report, as well as the pathology of the remainder of the core if different. This allows the technique to be assessed as a predictor of the pathology of the core and maybe as a guide to taking more accurate core-cut biopsies.

b) Examination of tumor bed:

Current trends in breast cancer care tend towards more conservative surgery where less breast tissue is removed. This is marked by the decrease in the number of mastectomies performed (removal of the whole breast) and an increase in the number of wide local excisions where the tumor is removed with between 1 and 2cm of normal breast tissue as a margin on all sides. During such wide local excision of a breast cancer, it is often difficult for the surgeon to identify the tumor margins just by direct inspection of the surgical wound. In this situation, biopsies are taken to see if there is still cancer present. These can be taken in the form of random biopsies from the quadrants of the cavity left after excision of the tumor, or as a set of cavity shavings where strips of tissue are removed from the walls of the cavity and sent separately for examination. When this was required, optical measurements were taken of the same site immediately prior to the conventional biopsy and the results correlated.

c) Sentinel and other axillary lymph nodes:

All breast cancers have the ability to metastasize to lymph nodes (glands) in the axilla. Knowledge of whether or not this has occurred is important in determining the stage of the cancer i.e. degree of spread. This gives an indication of a patient's prognosis and may determine if adjuvant treatment such as chemotherapy is warranted. The sentinel node is the first lymph node that drains the region of the breast containing the tumor and is now recognized as a marker for axillary lymph node involvement with cancer. If the sentinel node is clear of cancer then the chance of any other node being involved with cancer is extremely small [1].

The sentinel node can be identified by various dye labeling techniques including blue dyes that can be observed during surgery and colloids containing radioactive tracers that can be followed with a gamma ray probe. The optical absorption of the dyes must be taken into account when examining the spectrum of the lymph nodes. Once found and removed the sentinel node must be assessed histologically either by frozen section while the patient remains anesthetized, or later, by paraffin embedded section. If the node is involved with cancer, then the other nodes in the axilla need to be removed. It would be simpler if the other nodes could be removed at the same operation, but this is only possible if one can get a rapid answer on whether or not the sentinel node is involved. The optical biopsy could provide this answer. We are now taking optical measurements on sentinel nodes as well as other resected nodes, immediately after surgical excision and correlating the results with subsequent conventional histology. Further details of the methods are given in previous reports and published papers. The protocol for examining the nodes once excised was to bivalve the node, by cutting along the longest axis

without completely separating the two halves. In the case of the nodes identified as sentinel impressions of the node were taken for imprint cytology by pressing the bivalved node several times onto a glass slide. This slide would be dried, sent to the lab and examined by a cytologist. Although this procedure was being performed as part of a separate study it enabled the optical measurements taken on the node to be compared to an additional conventional diagnostic technique. Optical measurements would be taken typically from four points on the bivalved surface of the node, typically representing the center of the node, or medullar and the periphery of the node, or cortex. This would be done on both bivalved surfaces, typically with two spectra per point giving a total of eight spectra and four points per node examined.

Data collection, recording and analysis:

All optical data collected from patients was automatically recorded on a laptop computer, which runs the optical biopsy system. This is backed up onto disc after each new data set is collected. The raw data is transferred into a spreadsheet file along with the corresponding histological diagnosis. These files are used for analysis in both Los Alamos/Boston and London. Examples of the spectra obtained have been given in previous reports. In addition to the automatically recorded details, a paper pro-forma listing the position and clinical diagnosis of the spectra acquired was taken at the time of the procedure and later transferred into an electronic database. This allowed not only the histological (definitive) diagnosis but also the clinical suspicion to be compared to the recorded spectrum.

Development of spectral classification methods:

Initially two different automated methods of spectral classification were used to assess the degree of correlation between pathology and spectral pattern differences: artificial neural networks and hierarchical cluster analysis. Artificial neural networks (ANNs) were selected for study because of the expectation by our group and other researchers [2, 3] that ANNs would prove to be a generally useful method of tissue spectral classification. ANNs are well suited for classification in systems where model-based classification is difficult. Such is the case with ESS spectra of breast tissue because of its remarkable heterogeneity of tissue types (comprising glandular, adipose, fibrous, tubular, connective and other tissue types) with consequent broad variability in optical scattering and absorption properties. Hierarchical cluster analysis (HCA) was selected for study as an alternative to the many approaches to classification that provide unbounded class regions (including linear discriminant analysis, regression analysis and ANNs). Further details of these approaches are given in our published papers and previous reports.

In the second year of the program while the analysis detailed above continued at Los Alamos, the analytical approach was expanded in London to concentrate on a model based analysis (MBA) approach where certain consistent features within the spectra are analyzed and accounted for. Such features include the hemoglobin concentration (as mentioned above in the section on experimental work), the presence of blue dye or an absorption spectrum for ß-carotene, which is only present in fat and not in tumors. In addition a number of invariant linear portions of the spectra were noted by careful observation and linear approximations could be found for these sections. The remaining part of the procedure is to break the spectrum

up into linear portions and using a process of line-fitting and repeated re-normalization reduce the raw input to a series of gradients, intercepts and absorption co-efficients. When referenced to experimental measurements the absorption coefficients can be successfully converted to concentrations (or saturations in the case of Hemoglobin). The reduced set of descriptors (~30 as opposed to 1800 values for the whole raw spectrum) could then be fed as inputs into an appropriate neural net or hierarchical cluster model, for convenience, and to avoid repetition of effort between London and Los Alamos, the analysis of the model based results was done with a linear discriminant based method using the package SYSTAT. This pre-processing could result in a dramatic reduction in overall processing time and also the ability to get relative values between different spectra at certain fixed points.

In the third year of the program, continuing work on the MBA approach led to a patent application for the spectral decomposition algorithms, which was submitted in February 2001 in the United Kingdom and subsequently in the United States. A completed form DD 882 in respect of this invention was submitted on December 15, 2000. Investigations are currently underway as to appropriate ways to commercialize the technology and the advances made as a result of this program.

Data analysis results:

Data sets are now available from a total of 214 patients recruited in London; this includes 17 patients recruited in a preliminary study. Data from these patients yielded a total of 1250 matched pairs of optical and conventional histopathalogical data. This exceeds the totals anticipated in the revised statement of work. Although an average of 2 or 3 spectra were taken from each tru-cut or tumor bed site there is an excellent correlation between successive spectra taken from the same site, shown statistically by r^2 values for the cross correlation between separate spectra of >95% (>99% in the majority of cases). This shows consistency in the readings and allows multiple spectra from a single site to be averaged, further reducing the noise level. In the case of spectra acquired from different points on nodes the correlation is not so close so data acquired at different points had to be counted separately but with the same histological diagnosis. The in vivo sites studied included 100 percutaneous biopsies (TRU-CUT) and 147 tumor margins examined during surgery. Ex vivo studies included 127 specimens of breast tissue examined immediately after surgical removal and 264 from axillary nodes including 56 from nodes identified as sentinel nodes, also examined immediately after excision. Some measurements have been taken on exterior tissue to look at the possibility of identifying Paget's disease of the nipple in-situ. Currently this part of the investigation is at an early stage and although the diseases are related the spectra are not suitable for inclusion in the breast cancer analysis.

Because of the serious and prolonged technical problems encountered with the optical biopsy hardware and data collection at Little Rock it has been decided not to attempt to use any of the data collected there.

In total we have over 4500 reliable spectra from the 1250 sites mentioned above. The conventional histology showed that the breast tissues examined included evidence of 79 invasive cancers, 9 showing carcinoma in situ, a couple only showing predominantly atypical

ductal or lobular hyperplasia and 34 with benign breast disease (including fibrosis, sclerosing adenosis and other non malignant lumps). The rest showed a mixture of normal fibrous, fatty or lobular breast tissue. In the lymph nodes, 56 data sets showed metastatic cancer including two that showed micro-metastases, 69 displayed reactive changes while the remainder were examples of normal lymphatic tissue.

For all analyses the spectra from breast tissues and sentinel nodes were examined separately since they are basically different tissues. For the modes of analysis used at Los Alamos the spectra were pre-processed by first normalizing each spectrum to the same total integral over the spectral range of 350 to 750 nm ensuring that only spectral shapes were compared and not total scattering efficiencies. For the London analysis the pre-processing routines were more complex and some aspects have been described above.

Sensitivity and specificity are defined in the standard way:

$$\text{Sensitivity} = TP/(TP + FN) \qquad \& \qquad \text{Specificity} = TN/(TN + FP)$$

Where TP, FP, TN and FN represent the numbers of true positives, false positives, true negatives and false negatives, respectively, as determined by the corresponding histopathology. For the training and testing of the ANN's we randomly chose 80% of the data samples as a training set, reserving the remaining 20% as the test set. This was repeated three times with three different random choices of the 80/20 split. Results from the three different types of analysis for detecting cancer in both breast tissue and lymph nodes are shown below:

Table 1: Sensitivity and specificity for detection of cancer in breast tissue

	ANN	HCA	MBA
Sensitivity	**69%**	**67%**	**94%**
Specificity	**85%**	**79%**	**92%**

Table 2: Sensitivity and specificity for detection of cancer in axillary lymph nodes

	ANN	HCA	MBA
Sensitivity	**58%**	**91%**	**84%**
Specificity	**93%**	**76%**	**87%**

ANN: Artificial Neural Network, HCA: Hierarchical Cluster Analysis and MBA: Model Based Analysis

Serial changes in spectra with time after removal of tissue from the body

When tissue is removed from the body, the level of oxyhemoglobin will inevitably fall and the level of deoxyhemoglobin will rise. However, we undertook some further studies to see if the spectral features associated with normal tissue or malignancy would be preserved. Spectra taken from breast tissue in vivo were compared with spectra taken from exactly the same sites (marked by suturing a small cannula to the relevant site before the tissue was excised) after the tissue was removed from the patient. Serial measurements were taken over a period of one hour after excision with the tissue either at room temperature in air, at room temperature in

normal saline or kept on ice. In axillary nodes (from which spectra could not be taken in vivo), serial measurements were taken from the earliest possible moment after removal (typically less than 5 minutes) for the same period of time, although some specimens were put into formalin after the first measurement had been taken and serial measurements taken over the following 24 hours.

These studies were undertaken near the end of the research period and full analysis of the results is not yet complete. Nevertheless, preliminary analysis suggests that the serial changes seen are remarkably small and that the spectral features that best characterize the tissue are preserved. These results are important as it means that there is plenty of time available to take spectra from freshly excised tissues and that the most important information will not be lost in the first few minutes. In addition, the results in formalin open up the interesting new possibility that pathologists may be able to use the optical biopsy technique to assess fixed tissues to identify which areas are likely to reveal the most useful information when examined histologically.

Complementary studies of scattering from cell suspensions:

Under independent funding from a NCI/NIH grant, Dr. Judith R. Mourant, a colleague of Dr. Bigio at Los Alamos, has been studying the intrinsic scattering properties of parallel cell lines (cancerous and non cancerous). These studies demonstrate that the optical geometry of the fiber probes, as utilized for our breast cancer study, accentuates sensitivity of the collected scattering spectrum to variations in nuclear size and density, which are associated with malignancy [4, 5].

Complementary studies providing further optical biopsy data:

Under separate hospital funding in London, Dr Laurence Lovat, a colleague of Prof Bown, has used the flexible optical probe to make optical measurements endoscopically on a range of lesions in the gastrointestinal tract that required a conventional biopsy, so the optical and conventional biopsies could be correlated. This study is showing particular promise in identifying areas of pre-malignant change in Barrett's esophagus, which cannot be seen by conventional endoscopy alone. This is an increasingly important problem as the incidence of cancers in this site is increasing faster than for any other solid organ. To date a total of over 1100 measurements have been obtained from a group of over 230 patients and the initial results are looking extremely promising. The preliminary results identifying dysplasia within areas of Barrett's esophagus are given in table 3.

Table 3: Sensitivity and specificity for detection of pre-cancerous changes in Barrett's esophagus

		Elastic Scattering Spectroscopy	
		Dysplasia	**No Dysplasia**
Histology	**Dysplasia**	**16**	**2**
	No Dysplasia	**10**	**50**

Sensitivity	**89%**	**Specificity**	**83%**

A second study, in conjunction with the department of dermatology at UCL, has also been started where optical measurements are taken from suspicious pigmented skin lesions, 122 so far, which are undergoing surgical excision for histological analysis. The aim of this study is to develop an algorithm that will allow discrimination between benign and dysplastic naevi and malignant melanoma. This would have huge potential as a method of gaining immediate diagnostic information of any suspicious pigmented lesion so reducing the need for surgical biopsy. Analysis of the spectra from the first 76 lesions, for which we have optical and conventional histology results, shows a sensitivity of 73% and specificity of 93% in distinguishing malignant melanoma from benign naevi.

Similar measurements are being made in the mouth, particularly in patients known to have dysplasia or early cancer at one site, to see if such changes are present at other sites and at bronchoscopy in patients with dysplasia of bronchial mucosa. In these organs, there are not yet enough data to draw any conclusions, although there is considerable enthusiasm amongst our colleagues to explore the possibilities.

Comment:

The results on breast tissue so far continue to be very encouraging and we hope that as the number of measurements increases, we will be able to consolidate and confirm the promising sensitivity and specificity found so far. A lot more work is required to find the best way to analyze the spectra we have. We need to identify the features that best distinguish between normal and malignant tissue and to find as simple a way as possible to interpret the results to minimize the computing time required. The model-based analysis developed over the last couple of years seems a lot simpler than the artificial neural networks and hierarchical cluster analysis used in the first year and is giving comparable results, so this will be explored further. The hope is that an algorithm can be developed for rapid analysis of spectra so that in the clinical setting, the technique can be used to give almost immediate discrimination between benign and malignant tissue with a reliability that is comparable to conventional histology.

Objective 2: Improve treatment of breast cancer by using ILP.

2A: Detection of residual cancer after ILP by contrast enhanced MRI:
2B: Real-time, dynamic monitoring of ILP in an interventional MR scanner:

Pilot studies for these 2 objectives were undertaken prior to commencement of the current program, although since then various practical and administrative problems have been encountered, which have seriously hindered patient recruitment. The patients proposed for this study were those with a proven small (up to 2cm) carcinoma in the breast who were scheduled for routine surgery (wide local excision or mastectomy). Further inclusion criteria were no previous radiotherapy or surgery to the lesion to be treated and no contraindication to MR imaging contrast agents.

Details of the techniques proposed for this part of the study were given in earlier reports, (6,7,8) but as explained in the last report, we have been unable to recruit any patients. Several other research programs on early breast cancer started at the same time as this one resulting in

competition for recruitment of suitable patients. We tried to tackle this by making arrangements for appropriate patients to be identified in other hospitals and then referred to us, but we still failed to obtain any usable data.

2C: Long term healing after ILP to benign fibroadenomas of the breast.

This study was undertaken to understand how laser treated areas in the breast heal as it was felt inappropriate to leave laser treated cancers in the breast for an extended period of time at an early stage of this project. The technique of treatment was essentially the same as for interstitial laser photocoagulation of breast cancers, but most of the imaging was done using ultrasound rather than MRI. This was done because fibroadenomas are much easier to define on ultrasound than MRI, so the simpler and cheaper imaging option was chosen and further, if part of the lesion was missed or inadequately treated, it would not have the same serious consequences for the patient as if part of a cancer was missed.

The patients included in this study were those with palpable breast lumps confirmed to be benign fibroadenomas on clinical examination, ultrasound scan and fine needle aspiration. Some of the patients included in this study were first treated prior to the start of the present grant but their follow up was completed as part of the current program. Technical details are given in the publication "Interstitial Laser Photocoagulation for Fibroadenomas of the Breast" which was appended to an earlier report. The key early findings (on the first 12 lesions scanned a year after treatment) were that the median reduction in lesion size after treatment, as measured on ultrasound, was 38% at 3 months, 60% at 6 months and 100% at 12 months. None of the lesions examined at one year could be detected clinically and only one was detected on ultrasound. The treatment was a simple day case procedure. The only complication of note was a minor skin burn seen in 3 patients early in the series and since the technique was modified to protect the skin by cooling, this has not occurred again.

In addition to the 24 patients in the first study, another 36 patients with fibroadenomas have been treated since the start of the program. A control group of 15 patients with a total of 27 fibroadenomas has also been identified to monitor for the natural history of the condition. The follow up period on all these patients is not yet complete, but the general trend of the results is very similar to that seen in the first group treated with ILP. Further follow up will be carried out by our breast surgery team.

The treatment of benign breast fibroadenomas with ILP has proved so successful and has been so well accepted by both surgeons and patients that it has now been introduced into routine clinical practice in the University College London Hospitals. It is offered to patients as an alternative to surgical excision, as although it can take a year or more to shrink the lesion fully, it is simpler and more convenient for patients. It does not involve a general anesthetic, so is considerably cheaper and frees up time for other procedures in the operating room.

One of the anxieties at the outset of this program for treating breast cancer was that laser treatment, even if completely successful, would replace a palpable cancer in the breast with a palpable lump of scar tissue. We thought this would cause patients considerable anxiety, as they would worry that the cancer was still there. We have been very encouraged to find from

this study on fibroadenomas that all the tissue necrosed by laser treatment appears to be resorbed without leaving any palpable scar tissue, although this can take up to a year. This will make ILP a more attractive option to many patients.

2D: Delayed surgery after ILP in patients over 65 years treated with Tamoxifen.

This study was undertaken as a first step to understanding how laser treated breast cancers heal if they are not removed by conventional surgery shortly after ILP. This group of patients was chosen, as treating them with tamoxifen to shrink the cancer for 3 months prior to surgery is an accepted treatment option in the United Kingdom. The technique for the laser treatment itself is exactly the same as for patients treated with ILP just before surgery. Treatment was planned to cover the entire tumor, but the main aim of the study was to see if MRI could detect incompletely ablated cancers, so it was desirable that in some cases, ablation should be incomplete. This was acceptable as all patients were being treated with tamoxifen. All patients had pre-ILP, interim (6 weeks) and pre-surgery (3 months) MR scans (contrast enhanced) and the scans were correlated with the histological findings on the surgical specimen.

Results:

The number of patients suitable for this study was quite small and it was only ever planned that this should be carried out in London. To date, 8 patients have been recruited for ILP and so far 4 have had surgery 3 months later. In the surgical specimens, 2 patients had residual tumor. In one patient this was detected as a 2mm area of increased enhancement on the pre-surgery scan. As the specimens are orientated in the transverse plane, we were able to confirm that the abnormality on the MR scan corresponded exactly with the abnormality seen on histology. In the second patient, there was minimal enhancement on pre-operative scans so attempts to identify the extent of residual tumor on MR after treatment were unsatisfactory.

Examples from patients with small, ILP treated cancers:

PATIENT 1

Age:	68
Size of tumor:	10 x 10 mm
Core biopsy:	Grade 1, invasive ductal carcinoma.
No. of ILP needles:	1

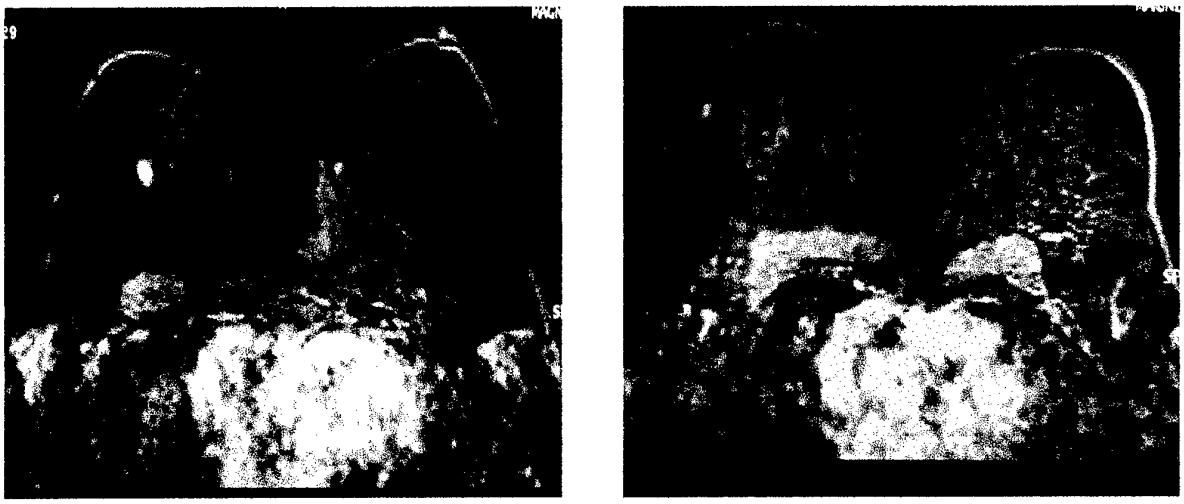

Figure 1 – Pre and Post laser MRI scans showing signal loss within the region of the original tumour with a surrounding rim of enhancement.

MRI findings: Initial scans pre-ILP demonstrated a well-defined enhancing tumor consistent with the mammographic findings of a small carcinoma. The pre-surgery scans revealed complete loss of signal from the tumor site with a surrounding rim of enhancement, interpreted as complete tumor destruction with a surrounding inflammatory response (Fig 1).

Histology findings: Breast tissue measuring 70 x 60 x 20 mm. On sectioning there was a well circumscribed yellow and brown lesion measuring 25 x 20 x 15 mm. Microscopically, the specimen revealed an area of coagulative necrosis surrounded by an area of fat necrosis. No viable tumour was identified within the specimen. Four axillary lymph nodes from sampling showed reactive change only with no evidence of metastatic carcinoma.

PATIENT 2

Age:	84
Size of tumor:	21 x 19 mm
Core biopsy:	Grade 2, mucinous carcinoma.
No. of ILP needles:	2

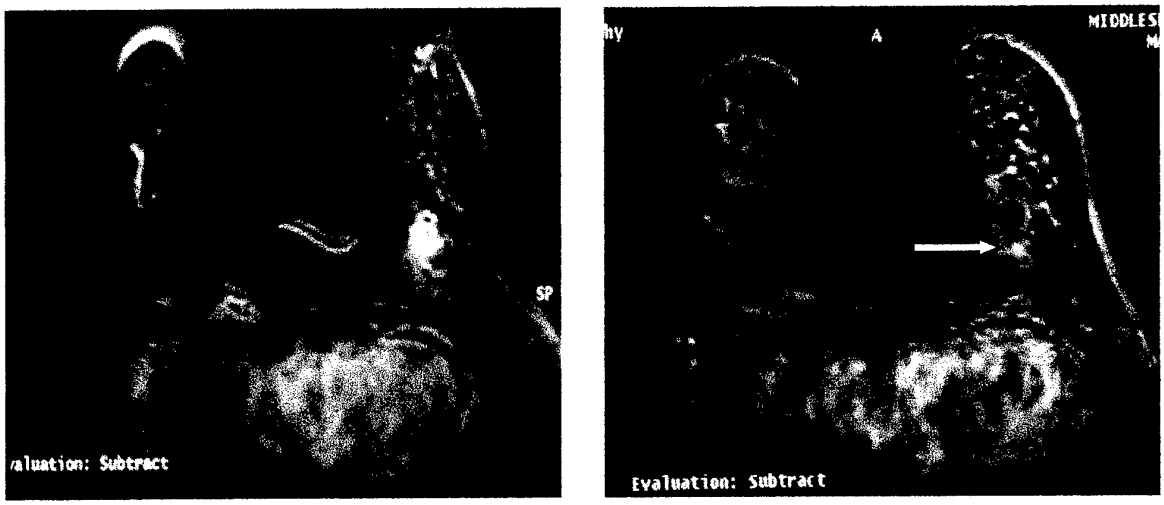

Figure 2 – Pre and Post ILP MR scans suggesting small area of residual tumor (arrow)

MRI findings: Initial scans revealed an irregular enhancing tumor adherent to the chest wall musculature. Pre-surgery scans showed loss of signal within most of the tumor but a small area of enhancement remained (2mm) (Fig 2). This was felt to be residual tumor.

Histology findings: Mastectomy specimen measuring 190 x 130 x 30 mm. On sectioning there was a lesion measuring 45 x 25 x 20 mm. Microscopically, there was an infarcted mucinous carcinoma. The majority of the tumor showed evidence of coagulative necrosis, although there were pockets of morphologically viable tumor, the largest measuring 2 mm. None of 12 excised lymph nodes showed evidence of metastatic carcinoma.

Comment:

The number of patients recruited on this protocol was small, but it is encouraging that a residual cancer as small as 2mm could be detected and that no areas of viable cancer were found on the surgical specimens that had not been seen on the MR scans.

KEY RESEARCH ACCOMPLISHMENTS

Part 1: Optical diagnosis of breast cancer:

a) Optical probes and associated instrumentation have been developed for the acquisition of reliable and reproducible elastic scattering spectra from breast and other tissues *in vivo* and *ex vivo*. Tissues retain their key spectral signatures ex vivo, even after some processing.

b) Spectral analysis gave a sensitivity up to 94% and a specificity up to 92% for detection of cancer in breast tissue (84% and 87% respectively for detecting metastases in excised axillary nodes). Larger data sets and improved diagnostic algorithms may improve these figures further.

c) Parallel studies in other tissues gave comparable results - sensitivity of 89% and specificity of 83% for detecting dysplasia in Barrett's oesophagus, 91% sensitivity and 79% specificity for detecting malignant melanoma in pigmented skin lesions.

d) Elastic scattering spectroscopy (ESS) has been shown to have considerable promise for the immediate detection of cancer *in vivo* in breast and other tissues without the need to remove tissue from the patient, as well as for detecting cancer *ex vivo* in excised tissues such as axillary nodes.

e) This straightforward, low cost technology could reduce the need for some routine biopsies as well as identifying the most appropriate sites from which conventional biopsies should be taken.

Part 2: Optical treatment of breast cancer: Interstitial Laser Photocoagulation (ILP):

a) Treatment of lesions in the breast by interstitial laser photocoagulation (ILP) is a simple and safe procedure that does not require general anesthesia and that leaves no scars. ILP induced areas of necrosis heal by resorption of the dead tissue over a period of months without leaving a fibrous lump.

b) This study was designed to assess the potential of ILP for treating small breast cancers, but the observations on the response of fibroadenomas were so impressive that ILP has now been introduced in our hospital as a routine treatment for these lesions. This treatment is particularly attractive to groups such as black women who are prone to keloid formation at the site of surgical scars, although no young woman wants a scar on her breast if it can be avoided.

c) Complete ablation of small, localized breast cancers with ILP is possible. Residual tumor left after ILP as small as 2mm in diameter can be detected on contrast enhanced MR scans. However, it will be necessary to get much more data on MR imaging of small, laser treated cancers, which are subsequently excised, before it will be ethically acceptable to start studies treating small breast cancers with ILP without subsequent surgical excision

d) Laser induced thermal destruction of cancers can be visualized by real time MR scanning, but it requires a high magnetic field, not generally available in interventional MR scanners (1T is adequate but 0.2T is not).

REPORTABLE OUTCOMES

Journal articles / Book chapters

1. Harms SE, Mumtaz H, Hyslop B, Klimberg S, Westbrook K, Korourian S: Magnetic Resonance Imaging Directed-Laser Lumpectomy. *Breast Diseases, A Year Book Quarterly,* St. Louis; Mosby. 1998.

2. LM Lai, MA Hall-Craggs, H Mumtaz, PM Ripley, TI Davidson, MW Kissin, C Saunders, I Taylor, SG Bown. Interstitial laser photocoagulation for fibroadenomas of the breast. The Breast 1999; 8:89-94.

3. Harms SE, Mumtaz H, Hyslop B, Klimberg S, Westbrook K, Korourian S: RODEO-MRI guided laser ablation of breast cancer. SPIE 1999; 3590:484-489.

4. C. D. O. Pickard, G. M. Briggs, L. Lovat, C. Saunders, P. M. Ripley, I. J. Bigio and S. G. Bown. Elastic Scattering Spectroscopy In-Vivo: Optical Biopsies of Cancers of the Breast and GI Tract. SPIE Proceedings Vol.3917

5. C. D. O. Pickard, G. M. Briggs, C. Saunders, P. M. Ripley, I. J. Bigio and S. G. Bown. Optical Biopsy: In-Vivo diagnosis of Breast Cancer using Elastic Scatter Spectroscopy. SPIE Proceedings Vol.3907, pp.592-599.

6. P. M. Ripley, D. Pickard, I. G. Rose, C. A. Kelley, I. J Bigio, G. Briggs, L. Lovat and S. G Bown. A Comparison of Artificial Intelligence Techniques for Spectral Classification in the Diagnosis of Human Pathologies based upon Optical Biopsy. OSA Biomedical Topical Meetings Proceedings, 2000, MC5

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Published Abstracts

Optical biopsies in the diagnosis of melanocytic lesions: comparison with clinical and histopathological diagnosis
J Scarisbrick, CDO Pickard, AC Lee, GM Briggs, SG Bown, MRS Keshtgar, RC Yu
British Journal of Dermatology 2002, 147 (Supplement 62): 7

Elastic Scattering Spectroscopy for the Diagnosis of Breast Cancer
AC Lee, CDO Pickard, MRS Keshtgar, GM Briggs, M Falzon, S Lakhani, PJ Ell, I Bigio, SG Bown *British Journal of Surgery 2002, 89 (Supplement)*

Intraoperative assessment by optical biopsy for lymph node metastasis in breast cancer
AC Lee, CDO Pickard, MRS Keshtgar, GM Briggs, M Falzon, S Lakhani, PJ Ell, I Bigio, SG Bown *British Journal of Surgery 2002, 89: 640-642*

Elastic Scattering Spectroscopy for Intraoperative Detection of Sentinel Lymph Node Metastasis
AC Lee, CDO Pickard, MRS Keshtgar, GM Briggs, M Falzon, S Lakhani, PJ Ell, IJ Bigio, SG Bown *European Journal of Cancer 2002, 38(Supplement 3): S59*

The Role of Dynamic Imaging in Sentinel Node Biopsy in Breast Cancer
AC Lee, MRS Keshtgar, WA Waddington, PJ Ell
European Journal of Cancer 2002, 38(Supplement 3): S60
European Journal of Cancer 2001, 37(Supplement 5): 17

Intra-operative assessment by optical biopsy for sentinel lymph node metastasis in breast cancer.
AC Lee, CDO Pickard, MRS Keshtgar, SG Bown, GM Briggs, S Lakhani, IJ Bigio, PJ Ell.
British Journal of Cancer 2001, 85(Supplement 1): 27

The role of intraoperative imprint cytology in determining the histological status of the sentinel lymph node.
MRS Keshtgar, A Lee, I Taylor, T Davidson, PJ Ell, D Ralphs, G Kocjan.
British Journal of Surgery 2001, 88(Supplement 1):

Presentations:

The Wendy & Emery Reves Internationl Breast Cancer Symposium October 1998, Dallas.
"RODEO MRI Guided Laser Lumpectomy: The potential for treatment without disfigurement". Harms S.

Radiology Society of North America (RSNA). Nov 1998, Chicago.
"Laser Lumpectomy with interactive MR imaging: Histopathological correlation". Harms S.

16th Annual Miami Breast Cancer Conference. Feb 1999. Miami.
"Integration of MRI and treatment planning". Harms S.

British Breast Group. May 1999 Edinburgh.
"Shedding Light on Breast Cancer". Saunders CM.

Biomedical Optical Spectroscopy and Diagnosis, (Munich, June 1999), OSA TOPS Vol. (1999, in press).
Invited: "Optical Diagnosis and Treatment of Breast Cancer," Bigio IJ. and Bown SG.

Interventional MRI , British Institute of Radiology. June 1999, London.
"Thermal Ablation of Breast Tumors". Briggs GM.

Light for Life, (Cancun, Mexico, July 1999), Springer Verlag (1999, in press).
Invited Plenary: "Minimally-invasive optical diagnosis and treatment of breast cancer," Bigio IJ. and Bown SG.

Advances in Optics for Biotechnology, Medicine and Surgery, 1-6th August 1999, Kona, Hawaii, USA.
"Diagnosis and Treatment of Breast Tumors through the combined use of Optical Biopsy and Interstitial Laser Photocoagulation". Ripley PM.

6th Nottingham International Breast Cancer Conference. Sept 1999, Nottingham.
"Lasers – A minimally invasive way forward for breast tumors". Briggs GM

Magnetic Resonance Radiologists Association. Oct 1999. London.
"Breast Biopsy and interstitial therapies". Hall-Craggs MA.

Breakthrough Breast Cancer meeting. Nov 1999, London.
"New methods of breast biopsy and minimal invasive techniques". Hall-Craggs M.
"Optical diagnosis of breast cancer". Bown SG.
"Local destruction of breast cancer with laser". Bown SG.

High Care 2000, Feb 2000, Bochum, Germany
"Optical Biopsy for Minimally-Invasive Diagnosis of Breast Cancer" Irving J. Bigio, Stephen G. Bown, Gavin Briggs, Christine Kelley, Sunil Lakhani, David Pickard, Paul M. Ripley, Ian G. Rose and Christobel Saunders

Era of Hope. June 2000, Atlanta.
"Interstitial laser photocoagulation for treatment of breast tumours". Oral presentation by Bown, S.G. and poster presentation by Briggs, G.M.
"Optical biopsy for the diagnosis of breast tumours". Pickard C.D.O.

British Society for Surgical Oncology, Nov. 2000, Nottingham
"Histological Assesment of Breast Tissue by Optical Biopsy" Briggs, G. M.
"Optical Biopsy for the Assessment of Sentinel Lymph Nodes of the Breast" Briggs, G. M.

Second International Congress on MR Mammography, Sept 2000, Jena Germany
"Laser Treatment in Benign Breast Disease" Hall-Craggs, M. A.
"RODEO MRI Directed Laser Lumpectomy" Harms, S.
"Minimally Invasive Therapy in Breast Cancer" Hall Craggs, M. A.
"Optical Biopsy of Breast Tissues" Bigio, I. J., Bown, S. G., Briggs, G. and Hall-Craggs, M. A.

European Conferences on Biomedical Optics, Munich, June 2001
"Optical biopsy for the diagnosis of breast tumours."
CDO Pickard, S Lakhani, IJ Bigio, SG Bown, GM Briggs, AC Lee, PM Ripley
"Interstitial Laser Photocoagulation of Breast Tumours"

1st Int. Conf. on Sentinel Node Biopsy in Mucosal Head and Neck Cancer, Jun 2001, Glasgow
"Optical Biopsy for the Assessment of Sentinel Lymph Nodes". Pickard et al.

Association of Surgeons of Great Britain and Ireland, Dublin, May 2002
Elastic Scattering Spectroscopy for the Diagnosis of Breast Cancer
AC Lee, CDO Pickard, MRS Keshtgar, GM Briggs, M Falzon, S Lakhani, I Bigio, SG Bown

3rd European Breast Cancer Conference, Barcelona March 2002
Elastic Scattering Spectroscopy for Intraoperative Detection of Sentinel Lymph Node Metastasis
AC Lee, CDO Pickard, MRS Keshtgar, GM Briggs, M Falzon, S Lakhani, PJ Ell, IJ Bigio, SG Bown

Society of Academic and Research Surgery 2002 Forum, London, January 2002
Intra-operative assessment by optical biopsy for lymph node metastasis in breast cancer
AC Lee, CDO Pickard, MRS Keshtgar, GM Briggs, M Falzon, S Lakhani, PJ Ell, IJ Bigio, SG Bown

7th Nottingham International Breast Cancer Conference, Nottingham, September 2001
The role of dynamic imaging in sentinel node biopsy in breast cancer
AC Lee, MRS Keshtgar, WA Waddington, PJ Ell

British Cancer Research Meeting 2001, Leeds, July 2001
Intra-operative assessment by optical biopsy for sentinel lymph node metastasis in breast cancer.
AC Lee, CDO Pickard, MRS Keshtgar, SG Bown, GM Briggs, S Lakhani, IJ Bigio, PJ Ell.

The 48th SNM Annual Meeting, June 2001
The Sentinel Node in Breast Carcinoma –Present Controversies.
MRS Keshtgar, A Lee, WAS Waddington

European Conferences on Biomedical Optics, Munich, June 2001
Optical biopsy for the diagnosis of breast tumours. CDO Pickard, S Lakhani, IJ Bigio, SG Bown, GM Briggs, AC Lee, PM Ripley

Association of Surgeons of Great Britain and Ireland, Birmingham, April 2001
The role of intraoperative imprint cytology in determining the histological status of the sentinel lymph node.
MRS Keshtgar, A Lee, I Taylor, T Davidson, PJ Ell, D Ralphs, G Kocjan.

Presentations to be made

Optical Biopsy: The Technique and Experience in determining Lymph Node Status in Breast Cancer GM Briggs, AC Lee, CDO Pickard, JR Sainsbury,MR Falzon, I Bigio, PJ Ell, SG Bown, MRS Keshtgar.
British Association of Surgical Oncology Meeting, London, November 2002

Clinical and physical performance assessment of a self contained, hand-held gamma probe
AC Lee, MRS Keshtgar, R Sainsbury, WA Waddington, PJ Ell.
Sentinel Node 2002, Yokohama, November 2002

Comparative Study of Imprint Cytology and Elastic Scattering Spectroscopy in assessing Sentinel Lymph Nodes status in Breast Cancer
AC Lee, M Keshtgar, CDO Pickard, G Kocjan, M Falzon, P Ell, S Bown, Bigio.
Sentinel Node 2002, Yokohama, November 2002

Optical Biopsy: The Technique and Experience in determining Lymph Node Status in Breast Cancer
MRS Keshtgar, AC Lee, CDO Pickard, G Briggs, M Falzon, SG Bown, PJ Ell, I Bigio.
Sentinel Node 2002, Yokohama, November 2002

Local Meetings

Pearce Gould Visiting Professor (Oncology Symposium), March 2002
The Role of Optical Biopsy in Breast Cancer Management

Tripartite Research Meeting, Middlesex Hospital, November 2001
Optical Biopsy in Breast Cancer

Pearce Gould Visiting Professor (Laser Symposium), March 2001
Optical Diagnosis with Elastic Scattering Spectroscopy Sentinel Node in Breast Cancer

Presentations on optical biopsy technique in organs other than the breast

British Association of Dermatologists' 82nd Annual Meeting, Edinburgh, July 2002
Optical biopsies in the diagnosis of pigmented lesions: Comparision with clinical and histopathological diagnosis

JJ Scarisbrick, CDO Pickard, AC Lee, GM Briggs, SG Bown, IJ Bigio, MRS Keshtgar, R Yu

British Society of Gastroenterology, 2000
Novel Optical biopsy technique using elastic scattering spectroscopy for dysplasia and cancer in Barrett's oesophagus.
Lovat LB, Pickard D, Novelli M, Ripley PM, Francis H, Bigio IJ, Bown SG.

Informatics:

Set-up of a computer database, (Microsoft Access) for patient records and a second for correlation of spectral data and histological findings. This includes patient details, treatment parameters, radiological and clinical follow-up, histology and any complications.

CONCLUSIONS

The work on optical diagnosis of breast cancer has proceeded extremely well. We now have a reliable system for taking optical readings at open surgery and through a biopsy needle. Preliminary analysis of the first sets of paired optical and histological data by artificial intelligence techniques showed reasonably high specificity and sensitivity for the detection of cancer in breast tissue and sentinel nodes. The work in the second year using model based analysis suggested that this simpler method may reduce the computing power required without jeopardizing the quality of the results, and further work in the 3rd year to refine this approach has resulted in a patent application. These results are encouraging, but more data are required to know how reliable these measurements are. The further data gathered over the last year of the project continued to expand the knowledge base of spectral signatures from diseased tissue and has helped to prepare a relatively simple algorithm for spectral analysis that can be run on a laptop or palmtop computer to give an immediate indication of whether a particular spectrum is from normal or malignant tissue. If successful, this technique may be able to provide immediate pathological information that is comparable to that provided by conventional histology. Although the main project has focused on breast and axillary nodes, other groups in the University College London Hospitals have gathered data from other tissues, especially Barrett's oesophagus and pigmented skin lesions, showing that the technique of elastic scattering spectroscopy has very considerable potential for the immediate detection of dysplasia and cancer in a wide range of tissues. We are currently seeking further funding to take this exciting work forward.

The studies on laser treatment of cancers have been seriously limited due to technical problems and difficulty recruiting patients. Nevertheless, it does seem feasible to completely ablate small cancers. Areas of viable cancer as small as 2mm in diameter remaining after ILP can be detected on contrast enhanced MR. We have shown that laser induced thermal changes can be detected in real time during laser energy delivery and that these may be able to predict when enough heat has been delivered to destroy the entire cancer, but current interventional MR scanners do not have a high enough magnetic field. ILP has the potential to provide a safe, relatively straightforward and effective treatment for selected small breast cancers that may have lower costs and better cosmesis than surgical lumpectomy. However, taking this work forward would require finding a hospital with the appropriate expertise and equipment (interventional radiologist and high quality MR scanner) as well as a suitable patient referral pattern to undertake the further studies required of ILP followed by imaging and surgical excision.

The long-term results from treating benign fibroadenomas have taught us a lot. The necrosed tissue is resorbed completely without leaving a residual lump of scar tissue in the breast and without any long-term complications. This is very reassuring, as necrosed cancer tissue is likely to be resorbed in the same way. If part of a fibroadenoma is inadequately treated, ILP can be repeated. It is also good for patients psychologically as if any women could feel a residual lump in the breast after ILP for cancer, she would worry that there was still some malignant tissue left behind, even if repeat biopsies showed no evidence of cancer. This particular study was undertaken to understand how ILP treated cancers could be expected to heal. However, the results have been so impressive that ILP is now offered as a routine treatment for benign fibroadenomas in patients who want active treatment but do not want

open surgical excision. This is a technique that could be taken up by almost any hospital. The only equipment required is a routine ultrasound scanner and a low power, infra-red, semi-conductor laser. This could be of particular importance to women who have multiple fibroadenomas or who have a tendency to keloid formation (like some black women) as the cosmetic result would be so much better than conventional surgery.

With the acceptance of the revised statement of work (March 2001) studies on laser treatment of cancers did continue, although very few patients could be recruited due to the withdrawal of Little Rock from the project and the fact that the existing problems of recruiting suitable patients in London were ongoing. This limited the amount of new information that could be generated on this aspect of the program, but the data that was gathered suggested that the technique is at least feasible.

"So what" section

The optical biopsy work has more than lived up to expectations. The equipment is quite low cost and straightforward, it can be used *in vivo* without removing tissue from the patient, or *ex vivo*, the results are approaching those of conventional histology and the potential is there for the results to be available almost instantaneously. Studies by colleagues in other specialties showed that similar results could be obtained in the esophagus and on pigmented skin lesions. This has the potential to become an important new diagnostic technique not only for breast tissue, but for a wide range of other tissues throughout the body. Further development and assessment should be encouraged as soon as possible.

The work using ILP to treat fibroadenomas was only undertaken to assess how laser treated breast tissue healed. However, ILP has proved so effective for treating these lesions that our hospital has adopted it as a routine treatment. It is a simple, safe, low cost and effective option that leaves no scars and which can be repeated, if necessary. We would encourage all breast surgery units to consider setting up such a service.

The work on treating small breast cancers with ILP was severely hampered by difficulty in recruiting suitable patients. Nevertheless, the data we do have suggests that the technique is safe and could be effective. If a hospital with a suitable referral pattern and the appropriate expertise and imaging equipment can be found, we consider that the study treating small breast cancers with ILP followed by contrast enhanced MR scanning prior to surgery, as in our original proposal, should be undertaken. We feel that ILP could still become a viable alternative to wide local excision for appropriately selected patients as part of the overall management of the disease, although a lot more data would be required before this could be justified in routine clinical practice.

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8. Cross, M.J., *et al.*, *New horizons in the diagnosis and treatment of breast cancer using magnetic resonance imaging.* American Journal of Surgery, 1993. 166(6): p. 749-53; discussion 753-5.

Appendix 1

List of Personnel Supported by DAMD

At University College London

Salary charged to US Army

Mr Gavin Briggs	Surgical research fellow
Mr Andrew Lee	Surgical research fellow
Dr Paul Ripley	Medical Physicist
Dr David Pickard	Medical Physicist

Contributed

Prof Stephen Bown	Principal Investigator
Miss Christobel Saunders	Consultant Breast Surgeon
Mr Mohammed Keshtgar	Consultant Breast Surgeon
Mr Richard Sainsbury	Consultant Breast Surgeon
Dr Margaret Hall-Craggs	Consultant Radiologist
Dr Sunil Lakhani	Consultant Histopathologist
Dr Mary Falzon	Consultant Histopathologist

At Los Alamos National Laboratory:

Prof Irving Bigio	Co-investigator
Dr. Judith R. Mourant	Research Scientist
Dr. Paul Ripley	Physicist (postdoctoral fellow)
Tamara Johnson	Technician
Christine Kelley	Student

At Boston University

Dr. Ousama A'amar	Research Associate
Fang Hui	Student
Jennifer Dobson	Student

Appendix 2

Published Articles

1. Interstitial laser photocoagulation for fibroadenomas of the breast
   LM Lai, MA Hall-Craggs, H Mumtaz, PM Ripley, TI Davidson, MW Kissin, C Saunders, I Taylor, SG Bown.
   The Breast **8:** 89-94 1999

2. Diagnosis of breast cancer using elastic-scattering spectroscopy: preliminary clinical results
   IJ Bigio, SG Bown, G Briggs, C Kelley, S Lakhani, D Pickard, PM Ripley, IG Rose, C Saunders
   Journal of Biomedical Optics **5(2):** 221-228 2000

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8. Optical Biopsy: The technique and experience in determining lymph node status in breast cancer
GM Briggs, AC Lee, CDO Pickard, JR Sainsbury, MR Falzon, I Bigio, PJ Ell, SG Bown, MRS Keshtgar
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10. Optical biopsy: The technique and experience in determining lymph node status in breast cancer
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ORIGINAL ARTICLE

Interstitial laser photocoagulation for fibroadenomas of the breast

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SUMMARY. Fibroadenomas are benign lesions of the breast that are common in young women. Most can safely be treated conservatively, but if they are not excised, the majority persist for several years and some increase in size. Interstitial Laser Photocoagulation is a new approach to treating these lesions. Under local anaesthesia and sedation, using ultrasound guidance, fine needles are inserted into the fibroadenoma and a laser fibre passed through each needle to coagulate the lesion in situ. Twenty-nine lesions (diameter 14–35 mm) have been treated in 24 patients. Twenty eight (97%) shrank in size on follow up ultrasound assessment. Fourteen were followed for a year, at which time none was palpable and only one could still be detected on ultrasound. In early cases there were three minor complications (a small scar at a site of needle insertion), later prevented by improved technique. This is a simple and safe outpatient procedure for ablating fibroadenomas that does not require open surgery.

INTRODUCTION

Fibroadenomas are benign lesions which develop from breast lobules at around the time of puberty, often becoming palpable a few years later. The peak incidence of these lumps is between the ages of 19 and 25 years. Fibroadenomas can be diagnosed with a high level of confidence using a combination of clinical examination, ultrasonography and fine needle aspiration cytology. Traditionally, surgical excision has been advocated for all palpable, discrete breast lumps because of the perceived risk of malignancy. However, as it is now appreciated that fibroadenomas are aberrations of normal breast development rather than true neoplasms,[1–4] conservative management is safe in women who are under 35 years of age and where there is confidence in the diagnosis.[3,5–8] As a consequence, the practice of routine excision of fibroadenomas is being increasingly questioned. Surgery for these lesions is usually straightforward, but still carries some risk of anaesthetic complications, wound infection, haemorrhage and scarring. In a largely young group of women, cosmesis is an important consideration and scarring may be unacceptable, particularly in the area of cleavage. This problem is exacerbated in patients who develop multiple fibroadenomas and those with a propensity to keloid formation. Furthermore, surgical excision of all benign breast lumps represents a significant workload, and a simple, non-surgical alternative would be an attractive, and potentially cost saving option.

Interstitial laser photocoagulation (ILP) is a technique first described in 1983 by Bown, whereby optical fibres inserted percutaneously through needles enable delivery of light directly into a lesion within a solid organ.[9] The mechanism of action is thermal, resulting in localized coagulation necrosis centred around the end of each fibre. As the treated area is in the centre of a solid organ, ILP requires concurrent imaging to localize the target lesion, guide the placement of optical fibres and assess the effect.[10] Initial studies of ILP in the human breast were performed using a single optical fibre inserted into cancers a few days prior to scheduled surgery and then examining the result in the excized tissue. This showed that areas of necrosis up to about 15 mm in diameter could be produced safely in the tumour and in the immediately surrounding normal tissue. The diameter of the zone of necrosis was made more predictable by a process of precharring the fibre tip prior to insertion.[11] More recently, in further studies of ILP on breast cancers prior to surgery, Mumtaz et al.[12] have shown that contrast enhanced magnetic resonance imaging (MRI) can define the extent of laser induced necrosis remarkably accurately.

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The aim of the present study was to evaluate the feasibility of ILP as a minimally invasive technique for treating fibroadenomas.

PATIENTS AND METHODS

Patients for this study were recruited from outpatient clinics at the University College London Hospitals and the Royal Surrey County Hospital, Guildford. All patients had palpable breast lumps proven on clinical examination, ultrasound and aspiration cytology to be benign fibroadenomas and were informed of the conventional management options of either removing the lesion surgically or just keeping it under observation. The first group of 16 patients had elected to have surgical removal of their fibroadenomas but were then told of the possibility of the new laser treatment and consented to have ILP whilst waiting for surgery. The second group of eight patients, recruited after encouraging results were found in the first group, were told of the possibility of ILP at the time their diagnosis was confirmed, and chose ILP as an alternative to surgery or observation. This study was approved by the ethics committees at both hospitals and all patients gave written, informed consent prior to their participation.

The fibroadenoma was first localized and its maximum diameter measured by ultrasonography (Aloka 650, 7.5 MHz probe, Aloka, Tokyo, Japan, or Siemens, 7.5 MHz probe, Siemens, Germany). ILP was performed using an aseptic technique under sedation with intravenous midazolam (2–5 mg) and pethidine (25–50 mg) and with monitoring for oxygen saturation and vital signs throughout the procedure. Initially, 10 to 15 ml of lignocaine 1% were infiltrated into the skin and the breast tissue around the fibroadenoma. Under ultrasound guidance, 1–4 19 G needles were then inserted percutaneously into the fibroadenoma (Figs 1 & 2). The tips of the needles were positioned 1 cm apart in the lump and a bare optical fibre loaded through each one. The tip of each fibre was precharred by heating its tip in a match prior to use as this had been shown previously to give more even tissue heating.[11] The needle and fibre were adjusted

Fig. 1 Application of Interstitial Laser Photocoagulation to a fibroadenoma of the breast.

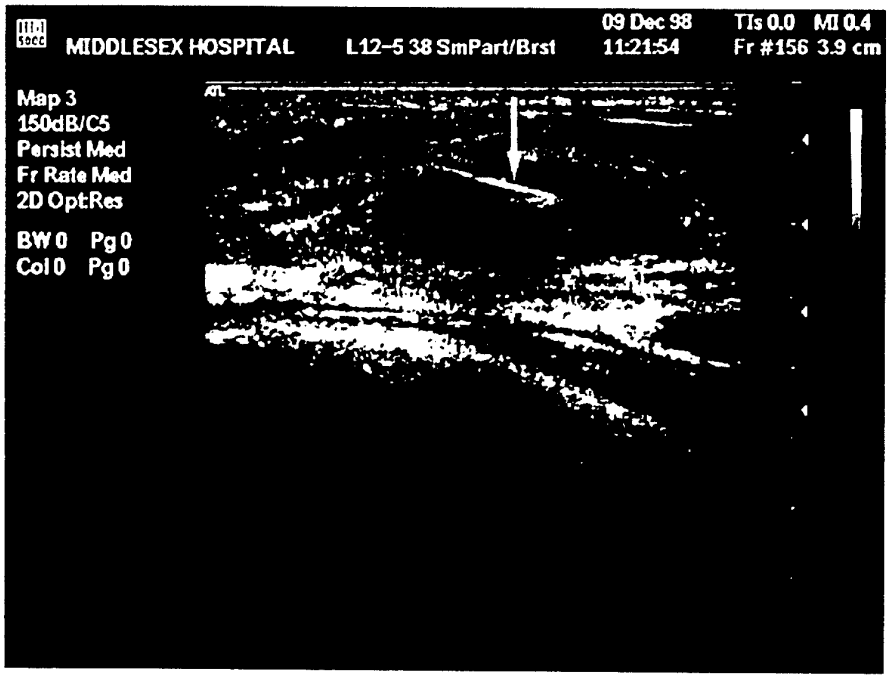

Fig. 2 Ultrasound scan of a laser fibre (arrow) positioned in a fibroadenoma immediately prior to ILP.

until the fibre protruded 4–5 mm from the needle, measured by marking the point on the fibre at which the proximal end of the needle had to be positioned. The number of fibres inserted was based on the size of the lesion to be treated (<15 mm: 1 fibre, 15–20 mm: 2 fibres, 20–25 mm: 3 fibres, >25 mm: 4 fibres). Once the fibres were in place, they were connected to a semiconductor diode laser (Diomed 25, Diomed Ltd, Cambridge, UK) via a beam-splitter (Diomed 4-way beamsplitter, Diomed, Cambridge, UK). This laser has a wavelength of 805 nm in the near infrared part of the spectrum, where tissue penetration is best for deep coagulation. The power used was 2.5 W per fibre for a planned duration of 500 s. When treatment was complete, the needles were removed and the patients allowed to go home as soon as they had recovered from the sedation.

Patients were seen for routine follow-up 2–4 weeks after treatment. The ultrasound scan was repeated at 3, 6 and 12 months after treatment. Any patient who was unhappy with the resolution of the lump in her breast at any time after ILP was offered surgical excision.

Seventeen of the patients treated in this series underwent contrast enhanced magnetic resonance imaging (MRI) on a 1.0T Magnetom 42SP scanner (Siemens, Erlangen Germany) before and after ILP to look for treatment induced changes. Transverse T1-weighted 3D Fast Low Angle Shot (FLASH) imaging was performed using the contrast agent gadopentate dimeglumine (Magnevist, Schering, Berlin, Germany) injected intravenously at a dose of 0.1 mmol/kg body weight. Post-ILP scans were performed 2–6 weeks after treatment.

RESULTS

A total of 24 patients with 29 proven fibroadenomas (2 patients with 2 lesions, 1 patient with 4) were treated. The median age of patients was 26 years (range 18–42 years) and the median size of lesions (measured by ultrasound) was 25 mm (range 14–35 mm). Twenty eight of the 29 (97%) lesions treated showed at least some reduction in size on serial ultrasound measurements. In one lesion there was no change. Only 6 of the original group of patients persisted with their original plan to have surgical excision following ILP, and 4 of these had surgery within a month of ILP, too short a time for there to be much reduction in lesion size due to the laser treatment. The other 2 (including the one with no change after ILP), requested surgery 12 and 24 weeks after ILP. None of those in the second group who chose ILP as an alternative to surgery have since requested surgery. Details of the lesions treated surgically are given in Table 1. All the excised lumps were confirmed to be fibroadenomas.

Table 1 Results on patients who underwent surgery after ILP

Patient number	1	2	3	4	5	6
Age	22	23	25	28	29	25
Size of fibroadenoma before ILP (mm)*	22	28	24	35	34	25
Time from ILP to surgery (weeks)	2	24	2	4	2	12
Size immediately prior to surgery (mm)*	18	14	16	28	27	25
Size of lump on histology (mm)	18	16	18	25	30	30
Residual viable fibroadenoma (mm)	3	6	3	3	2	16

*Fibroadenoma sizes were measured by ultrasound

For the patients who declined surgery, the results are shown in Table 2. The median reduction in lesion size after ILP, as measured with ultrasound, was 38% at 3 months, 60% at 6 months and 100% at 12 months. In all 14 patients so far followed up for at least 1 year, the treated fibroadenomas were no longer palpable. Twelve of these had ultrasonography at 1 year, but only 1 patient still had a detectable lesion. This had measured 33 mm at presentation and had shrunk to 10 mm at 1 year. No fibroadenoma increased in size (other than some oedema in the first few days after ILP).

Of the 17 lesions that were assessed with contrast enhanced MRI before and after ILP, 11 were depicted as discrete, strongly enhancing masses on pre-treatment images. Following ILP, there was no change in the 6 lesions that had not enhanced prior to treatment, but zones of persistent non-enhancement were demonstrated in the previously enhancing areas in all the other 11 cases. In 2 of these patients who subsequently had surgery, these new zones of non-enhancement corresponded to areas of laser induced necrosis seen histologically (Figs 3 & 4).

Most patients (n=20) reported feeling some discomfort

Table 2 Size of fibroadenomas measured by ultrasound prior to treatment and up to 12 months after ILP. The lesion seen on ultrasound at 12 months was not detectable clinically

Time	Lesion size (mm)		Percentage reduction in lesion size compared with size at presentation		Number of lesions examined	Number (%) of lesions no longer detectable on ultrasound
	Median	Range	Median	Range		
Prior to treatment	25	14–35	NA	NA	23	NA
3 months after ILP	14	0–23	38	5–100	23	1 (4%)
6 months after ILP	10	0–19	60	9–100	23	4 (17%)
12 months after ILP	0	0–10	100	71–100	12*	11 (92%)

*Two further treated lesions were undetectable clinically at 12 months but did not have an ultrasound scan at that time.

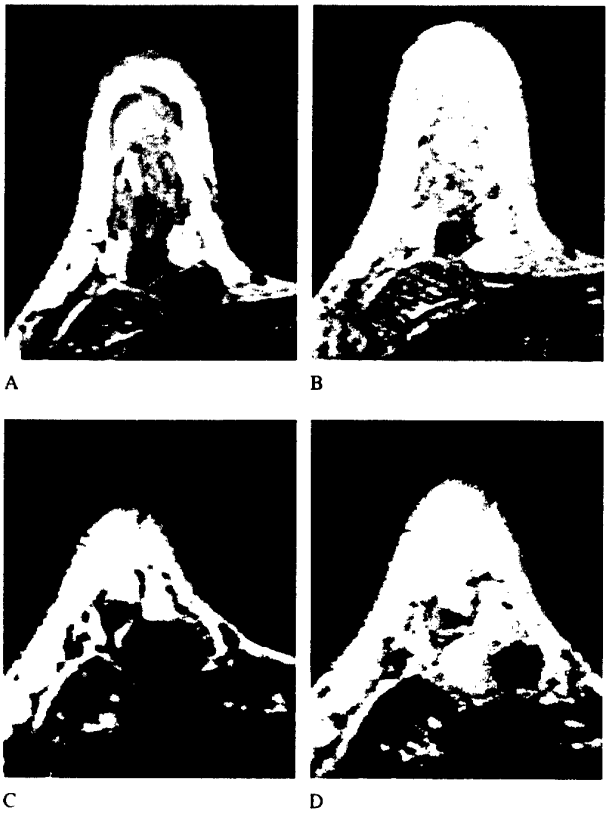

Fig. 3 MRI scans of fibroadenoma (patient 6 in the surgical group). (A) before ILP, without contrast; (B) before ILP, with contrast. (C) 3 months after ILP, without contrast; (D) 3 months after ILP, with contrast.

Fig. 4 Histological section of the fibroadenoma imaged in Figure 3, removed a few days after the MRI scans were taken.

whilst the laser was activated, but were able to tolerate the procedure satisfactorily with the use of intravenous analgesia and sedation in addition to the local anaesthetic in the breast. Four patients experienced more severe pain whilst the laser was activated and treatment was stopped prematurely after 261–300 s. Pain was relieved immediately when the laser was turned off. Even so, no treatment was stopped before at least half of the planned energy dose had been delivered and these patients still responded well. All patients were able to return to their normal activities the day after ILP. Local tenderness and swelling was reported by all patients following ILP. The swelling resolved by 1 week in most cases, although tenderness and sensitivity to touch in the treated breast persisted for anything from 1 day to 5 weeks (median 1 week). Bruising was seen in 4 patients but this resolved by 1 week in all cases.

There was no change in the size or shape of any of the breasts treated. Twenty-one patients had no visible scar following ILP to their fibroadenomas. The only complication was a small skin burn seen around a needle insertion point in three patients. In one of these there was also a persistent, clear, oily discharge for 3 weeks. Repeated cultures showed this to be sterile. These lesions all healed without further intervention. There were no wound infections and prophylactic antibiotics were not used. The resultant scars were all less than 1 cm in diameter, and all 3 patients felt these to be cosmetically acceptable.

DISCUSSION

Fibroadenomas are the commonest cause of discrete breast lumps in young women.[13] Although benign in nature, they are often a source of discomfort and anxiety. Since fibroadenomas can be diagnosed with a high degree of confidence on 'triple assessment' (clinical examination, ultrasonography and fine needle aspiration cytology) and conservative management has been shown to be a safe alternative to surgical excision, at least in patients up to about 35 years of age,[7] clinicians are more inclined to adopt a conservative approach in their management. Nevertheless, even if the conservative approach is safe, many fibroadenomas do not resolve. Dixon et al.[14] reported 152 patients with 163 fibroadenomas who were followed for 2 years. Thirteen lesions (8%) increased in size and were excised, 19 (12%) reduced in size, 42 (26%) resolved completely (20 (12%) at 1 year and 22 (14%) in the second year) and 89 (54%) did not change in size. Thus, of the 150 lesions treated conservatively, 130 (87%) were still present after 1 year and 108 (72%) after 2 years. Carty et al.[7] reported very similar figures. Dent et al.[4] reported that 31% resolve and another 12% get smaller. Wilkinson et al.[15] concluded that the majority of fibroadenomas continue to grow.

In contrast, in the present study, 14 treated lesions were followed up for 1 year, and all were impalpable on clinical examination at the end of this period. In the 12 patients that had ultrasound scans at 1 year, only one residual fibroadenoma could be detected. No sustained increase in size of any fibroadenoma occured after ILP. The transient swelling

seen shortly after treatment was attributable to inflammation and oedema around the treated area and settled within a week or so. These results compare very favourably with the less frequent spontaneous regression rates reported in the series treated conservatively. Seventy-five per cent of our patients who had initially opted for surgery decided not to proceed with surgery after ILP and were happy with the final result when interviewed at follow-up.

The major attraction of ILP is its potential for inducing resorption of fibroadenomas without leaving a surgical scar. Three patients sustained small skin burns which healed without complication. In one patient, this was due to placement of a fibre too superficially in a lesion just below the skin. The other two were 'needle tract burns' caused by retrograde charring of the fibre which caused heating of the needle shaft. These burns affected a small area (about 0.5 cm in diameter) around a needle track. In the patient who had a sterile discharge for 3 weeks, ultrasound scanning showed some liquefaction in the treated area, most likely from the necrosed tissue. In retrospect, the burns could probably have been prevented by more vigilant monitoring of skin temperature and checking that the fibre tip remained in the correct position, protruding far enough beyond the needle tip. Our current practice is to irrigate the area being treated with cold, sterile saline during laser activation, and no further burns have occurred since. The skin burns did not occur in any patient who complained of pain during the laser activation. This pain was most likely due to inadequate local anaesthesia. Even though these patients received little more than half the planned light dose, the biological effect was as good as in the other patients, which means that the treatment times used may have been longer than necessary.

Fibroadenomas have more clearly defined margins than most cancers. This makes them easy to delineate with ultrasound,[16,17] which has proved convenient for guiding fibre insertion and for monitoring the lesion size during follow-up. In the 6 lesions which were excised in this study, close correlation was observed between ultrasonographic measurements and histological measurements of lesion size (Table 1). A previous report[11] showed that ultrasound is poor at determining the extent of laser induced necrosis in real time or in the first few days after treatment, and we have not used it for this purpose in this study. However, unlike treating cancers, this is not critical for treating fibroadenomas, as the patient will not come to any harm if part of the lesion is left untreated. From our previous experience,[12] contrast enhanced MRI defines the extent of laser induced necrosis in cancers very well, and this was assessed in some of the lesions treated in this series. In the 11 lesions that enhanced on MRI prior to ILP, new zones of non-enhancement were seen after treatment and these were confirmed to be due to laser induced necrosis in two of the patients who opted for surgical resection after ILP (Figs 3 & 4). However, for routine management of these patients, MRI is not necessary as all the required imaging information can be obtained using ultrasound.

Patient acceptability of conservative management of benign fibroadenomas varies widely. In his series of 202 patients, Dixon et al.[14] reported that 92% initially opted for conservative management and only those with lesions increasing in size during follow-up were subsequently excised. Another UK series[15] reported more than 80% of women opting for conservative management, but a series from South Africa[8] reported that amongst patients who had had fibroadenomas excised, only 21% would opt for conservative management if they developed another lesion and only 7% would have preferred to keep their original lesion. In these series, patients were only offered either surgical excision or conservative management. In general, few patients will choose to have a surgical procedure that is not absolutely necessary. A simple procedure with no cosmetic sequelae may be more acceptable if it is shown to be safe and effective.

ILP is relatively simple to perform and the equipment required (a small, portable laser and a standard ultrasound scanner) is straightforward to set up and maintain. It is a day case procedure that can be done in the ultrasound department with local anaesthetic and sedation and which does not require full operating theatre facilities. Surgical excision of fibroadenomas is a relatively minor procedure with few complications, although operating theatre facilities are required, a general anaesthetic is usually preferred and there will always be a small scar, even though this can be well hidden.

In conclusion, in this pilot study, ILP has induced resorption of almost all the fibroadenomas treated during a follow-up period of a year during which time less than 20% would have been expected to disappear spontaneously. Patient satisfaction was high with this simple outpatient procedure. Ultrasound guided ILP has the potential to become an effective and low cost technique for managing fibroadenomas in patients who are anxious to get rid of their lump, particularly those with multiple lesions and those who have a tendency to keloid formation.

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Diagnosis of breast cancer using elastic-scattering spectroscopy: preliminary clinical results

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Abstract. We report on the first stages of a clinical study designed to test elastic-scattering spectroscopy, mediated by fiberoptic probes, for three specific clinical applications in breast-tissue diagnosis: (1) a transdermal-needle (interstitial) measurement for instant diagnosis with minimal invasiveness similar to fine-needle aspiration but with sensitivity to a larger tissue volume, (2) a hand-held diagnostic probe for use in assessing tumor/resection margins during open surgery, and (3) use of the same probe for real-time assessment of the "sentinel" node during surgery to determine the presence or absence of tumor (metastatic). Preliminary results from *in vivo* measurements on 31 women are encouraging. Optical spectra were measured on 72 histology sites in breast tissue, and 54 histology sites in sentinel nodes. Two different artificial intelligence methods of spectral classification were studied. Artificial neural networks yielded sensitivities of 69% and 58%, and specificities of 85% and 93%, for breast tissue and sentinel nodes, respectively. Hierarchical cluster analysis yielded sensitivities of 67% and 91%, and specificities of 79% and 77%, for breast tissue and sentinel nodes, respectively. These values are expected to improve as the data sets continue to grow and more sophisticated data preprocessing is employed. The study will enroll up to 400 patients over the next two years. [S1083-3668(00)00302-6]

Keywords: elastic-scattering spectroscopy; diffuse reflectance; tissue spectroscopy; optical diagnosis; optical biopsy.

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1 Introduction and Background

Various types of optical spectroscopy have been investigated as methods of "optical biopsy," with a majority of research efforts focusing on UV-induced fluorescence or Raman spectroscopies. (For a review of the topic, see, for example, Ref. 1 or references contained therein.) Recently, elastic-scattering spectroscopy (ESS) (sometimes called "diffuse reflectance spectroscopy") has been studied as a method for minimally invasive optical diagnosis of tissue pathologies, with emphasis on distinguishing dysplasia and cancer from normal tissue or other benign conditions[2–4] or for distinguishing different normal tissue types.[5] ESS, when performed using an appropriate optical geometry,[6,7] is sensitive to the sizes and structures of the subcellular components that change upon transformation to premalignant or malignant conditions (e.g., the nucleus, mitochondria, etc).[8] In work reported to date, clinical application of the ESS technique has been fundamentally noninvasive, with access to the tissue mediated by endoscopes[2–4] or by means of direct topical access as would be appropriate for diagnosis on the cervix.[9,10]

Within solid organs, such as the breast, the least invasive approach requires access through a needle. In screening, most early breast cancers are detected when an abnormality is seen on mammography, although some patients may detect a lump themselves (palpation). In either case, before any treatment is initiated, the diagnosis must be confirmed by fine-needle aspiration cytology (FNA) or biopsy (which is often done as an open procedure). Approximately 50 000 diagnostic lumpectomies are performed annually in the U.S. Of those, only about 12 000 turn out to be malignant when histology is performed on the excised tissue by a pathologist.[11] If it had been known in advance that the remaining 38 000 lesions were benign, the potentially disfiguring surgery could have been avoided, along with the trauma and cost, as many benign lesions resolve spontaneously in time, without intervention. A core biopsy, through a large-gauge needle, is a frequent alternative to surgical resection or lumpectomy for biopsy. Although less invasive than core biopsy, FNA is only infrequently used in the U.S. because false-negative rates with FNA are often in the range of 12%–15%,[12,13] and its reliability is under critical review. This is due to the heterogeneity of breast lesions and the relatively small number of cells accessed by FNA. Despite having the same level of invasiveness as FNA, the ESS

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1083-3668/2000/$15.00

method poses the potential advantage of immediate diagnosis, and there is the possibility of improved sensitivity when compared with FNA, due to the larger tissue volume that is sensed by the ESS method. If the optical biopsy technique based on ESS proves sufficiently reliable, it gives an immediate result, which minimizes the time a patient must wait for a diagnosis and might make it realistic to treat a small cancer at the same clinic visit. This will reduce patient anxiety and reduce costs for the large number of patients who have suspicious areas seen on mammography. Image-guided optical biopsy either with magnetic resonance, ultrasound, or conventional mammography could prove particularly valuable for patients with more than one suspicious area.

However, two other applications of ESS diagnosis to breast cancer may provide even greater patient benefits than the transdermal needle measurement. For open surgery procedures, the first of these is the object of our effort to develop a probe for the surgeon to use during breast-conserving surgery (wide local excision or partial mastectomy) for determining the status of the resection margins (the tissue surface, or "tumor bed," that is left after the suspect tissue has been excised) in real time. In current surgical practice, especially when the tumor limits are not clearly visible, the surgeon (and anesthetized patient) are required to wait for pathology results on a frozen section of the excised tissue to determine if tumor-free margins of excision have been established. Immediate frozen-section pathology is not always available, and, in real practice, with delayed pathology positive margins are found in as many as 20%–55% of all breast-conserving surgeries,[14] requiring a second surgical procedure.

The other potentially important surgical application being investigated in this study is real-time assessment of a "sentinel" lymph node in the axilla. For years, there has been controversy about how the axilla should be treated in breast cancer patients. Recent research has shown that if the main node in the axilla draining a tumor area, called the sentinel node, is removed and does *not* show cancer, then the chances of any other nodes in the axilla showing cancer are approaching 1%.[15] Thus, if the sentinel node does not show cancer, the rest of the axillary nodes can be left in place, but if it does show cancer, then a full surgical axillary-node clearance must be done. The sentinel node can be identified by injecting a radioactive marker and then scanning the axilla about 24 h later (during surgical preparation) or by using a dye such as methylene blue, which can be easily detected in tissue during surgery. The ESS method may be able to provide immediate assessment of the sentinel node during surgery.

Our diagnostic study is coupled, utilizing the same patient cohort, with a study of minimally invasive optical treatment. In the case of focal lesions, treatment can be accomplished using interstitial laser photocoagulation with laser energy from a diode laser, again mediated by fiber optics through a transdermal needle, to nonsurgically treat both adenocarcinomas and fibroadenomas. Results for that part of the study will be reported in separate publication(s).

2 Materials and Methods

The clinical instrumentation based upon elastic-scattering spectroscopy is essentially similar to that described in publications on earlier clinical studies.[2,3] The system (see Figure 1)

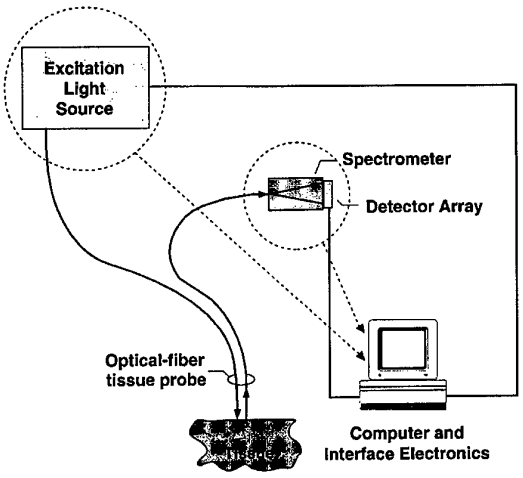

Fig. 1 Schematic diagram of the principal components of the ESS diagnostic system. All components (except for the fiber probe) are located inside a small, portable chassis.

consists of a pulsed xenon-arc lamp (EG&G) for the light source, a PC-compatible spectrometer, (a modified version of a spectrometer manufactured by Ocean Optics, Dunedin, FL), which employs a linear charge-coupled device (CCD) array for detection, an optical-fiber based probe, and a laptop computer for system control and data display. The wavelength range of the system is from 300 to 900 nm, but the range used for these studies is 330–750 nm, which covers the near-UV-visible part of the electromagnetic spectrum. As depicted in Figure 2, the probe is designed to be used in (gentle) optical contact with the tissue and incorporates two optical fibers, which are selected for their broadband light transmission over the spectral range used in the study. The output of the arc lamp is coupled to the illumination fiber, with a core diameter of 400 μm, which transmits the light to the tissue target site. A second, adjacent and parallel fiber within the probe, with a core diameter of 200 μm, collects a small fraction of the scattered light from the tissue. The collected light is then guided to the spectrometer where an optical spectrum is generated for further processing. For all of the measurements in this study, the probe design fixes the center-to-center separation of the collection and delivery fibers at 350 μm, at the tip of the probe. For this probe geometry, the volume of tissue

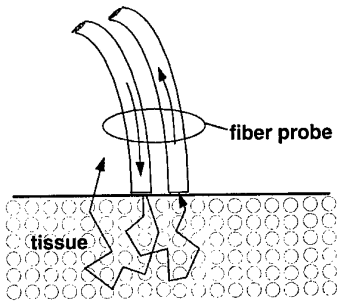

Fig. 2 Depiction of the optical geometry for the fiberoptic probe.

visited by the collected photons occupies a zone approximately 500 μm long, 300 μm wide and 300 μm deep. This has been determined from computational simulations using our Monte Carlo code, which incorporates Mie theory for the details of the scattering events.[6]

In clinical use the tip of the fiber probe is momentarily placed in contact with the suspect tissue, and the measurement is activated, at the keyboard or with a foot pedal. The system automatically takes a background (ambient+''dark'') measurement without firing the lamp, followed immediately (within 100 ms) by an ESS measurement with the pulsed lamp being triggered, and then subtracts the background spectrum from the ESS spectrum. The entire measurement process, i.e., activating the spectrometer, triggering the arc lamp, reading the detector array with an analog/digital (A/D) converter, etc., is computer controlled by a laptop PC, and can be activated with a foot pedal. This allows both accurate and reproducible measurements within the clinical setting. Furthermore, it also provides the clinician with the advantages of rapid data acquisition and a graphical display for inspection. Typical data acquisition and display time is less than 1 s for each site measurement. More specific details about the basic concepts of ESS, and discussion about the optical system and the design philosophy of the optical fiber probe, can be found in previous publications.[6,16,17]

Prior to any clinical measurements the ESS system and probe are calibrated with a reflectance standard. The reflectance standard (Spectralon™, Labspere, Inc., North Sutton, NH) has a spectrally flat, diffuse reflectance >98%, over the entire wavelength range of the system. The purpose of referencing the system to a known standard is to allow the normalization of spectral data against the overall system response. This technique effectively minimizes any variations in the system response due to variations in the spectral transmission among different probes, thermal effects, coupling efficiency of the fiber probe, drifts in detector/spectrometer response, etc. The reference standard and probes are sterilized by autoclave, and the housing of the reference standard protects it from any airborne contaminants during surgery.

Two different optical-probe configurations have been fabricated for use in the clinical program. Both probes incorporate the same ''standardized'' optical design, which specifies the diameters of the illumination and collection fibers, as well as the distance between the centers of the fibers, as specified above. It should be noted that details of the probe's optical design, including the fiber sizes and separation, have a significant effect upon the characteristic spectra obtained for a particular tissue. Therefore, these parameters are standardized for each clinical study in order to optimize the sensitivity of the system to tissue variations and also to prevent spectral variations due to instrumental artifacts. The main difference between the two probes designed for this study is to be found in the mechanical housing of the probe, allowing each to be optimized for the different specific clinical procedures. For interstitial (transdermal) measurements the probes have a small-diameter ($\leq$1 mm) stainless-steel outer sheath, which houses the optical fibers. The outer diameter of these probes was carefully chosen to be compatible with the inner bore of current core-biopsy needles in use at the clinics, so that a probe could safely and easily be passed down the needle and presented to the tumor tissue under investigation. The second probe design incorporates a larger stainless-steel sheath, ~5 mm in diameter, to house the optical fibers, with no change to the fiber dimensions or separation. This creates an ergonomically convenient, pen-like design, which is utilized, hand held, for optical measurements during open surgery, when both resected tumor margins and lymph nodes are addressed.

3 *In Vivo* Measurement Procedures

All clinical measurements reported here have been performed at the Middlesex Hospital, University College London Hospitals, UK. Histological examination of all tissue samples has been performed by the same pathologist (S.L.). Before any *in vivo* measurements were made, *ex vivo* tissue samples were obtained from resected breast specimens from 15 patients and were measured with the ESS system. These initial findings were used to optimize the operational settings of the system, and also helped to establish protocols for marking the optical biopsy sites for histological examination and correlation. All *in vivo* measurements are performed under a protocol approved by the Ethical Review Board of the Middlesex Hospital. Prior to surgery every patient within the study is informed about the research program, and informed consent for data to be taken is provided by the patient. As mentioned in the previous section, three separate areas are currently under investigation, and each warrants discussion due to the techniques employed in order to obtain the ESS spectra.

For interstitial measurements with the ESS system, a core-biopsy (Tru-cut™) needle is guided into the palpable lesion, sometimes with the aid of ultrasound or magnetic-resonance imaging. The appropriate ESS optical probe is then inserted through the needle and gently placed in direct contact with the tissue at the needle's distal end. After the optical measurement has been made, the fiber probe is withdrawn, and the inner component of the biopsy tool is reinserted and a core biopsy is then taken from the same site, without moving the needle. This produces a small worm-shaped biopsy sample, one end of which has been measured optically. The other end of the core sample (which is opposite to the end of the sample that was interrogated by the ESS probe) is marked with India ink so that proper orientation is preserved and identification of the end examined with the ESS probe can be maintained and correlated with histology.

Following the core-biopsy procedures, some of the patients immediately undergo surgery in order to have the tumor resected. In these and other surgical cases (not preceded by core biopsy), and depending upon the exact nature of the surgical procedure, measurements are taken from one or more locations of the tumor bed, during and immediately after resection. Each optical measurement is followed by a small surgical biopsy of the same site, and the biopsy samples are coded to correlate with the ESS probe measurements.

With a subset of the surgical patients, assessment of the sentinel node is performed in order to determine the presence of metastatic disease. (In a number of patients more than one node was assessed.) During the surgical procedure the sentinel node is located with the aid of radioactive tracer and/or blue dye (both injected previously into the tissue space near the tumor). The node is resected and cut in half, and ESS measurements are made on one or more sites on the cut surface of the node. Once again, the ESS measurement sites are

Table 1 Histopathology of optically measured tissue sites.

	Number of patients	Number of biopsies	Number of cancer sites	Number of normal sites
Breast tissue	24	72	13	59
Sentinel nodes	21	54	12	42

indicated to assist the pathologist in providing the corresponding histopathology information about the specific locations measured.

4 Methods of Classification

The data reported here were taken on the first 31 patients of the study (which will eventually enroll up to 400 patients over the next two years), with breast-tissue measurements (either as an interstitial measurement or during surgery) being made on 24 of them and lymph-node measurements (during surgery) on 21 of them. Thus, both breast-tissue measurements and sentinel-node measurements were made on many, but not all, of the patients. The numbers of patients and measurements are summarized in Table 1. The last two columns list the histopathology designations for the sites measured optically, i.e., the number of true positives and true negatives. In many cases two or three optical measurements were made for a given biopsy site to test repeatability of the spectra. However, it is not appropriate to treat multiple spectra from the same biopsy site as independent measurements, as this could result in incorrectly high *or* low sensitivity and/or specificity. Consequently, in such cases the multiple spectra (which were very similar) were averaged together and treated as a single spectrum. Thus, one spectrum is correlated with each biopsy site. An effort was always made to mark precisely each site of optical measurement, so that corresponding histopathology could be properly correlated.

Figures 3 and 4 show examples of tissue spectra, malignant and nonmalignant, for breast tissue and sentinel nodes, respectively. The spectra in Figure 3 are examples that are representative of some but not all of the data. It would be misleading to assume otherwise, given the heterogeneity of breast tissue (glandular, adipose, connective, fibrous, ductal structures, etc.) and the resulting large spectral variations. Consequently, it would be risky to base diagnosis directly on a model for any specific/common signature causes. For example, Figure 3 does not show a difference in hemoglobin saturation between normal breast tissue and ductal carcinoma *in situ* (DCIS), although there is evidence of more perfusion in the DCIS, which is expected. (On the other hand, Figure 4 does indicate some desaturation for the metastatic sentinel node, and the spectra are more representative of other node spectra, since nodes are less heterogeneous.) Reduced saturation *can* indicate cancer indirectly because necrotic regions are more likely to be cancer than benign, but DCIS is an early, pre-invasive stage, and does not generally invoke necrosis. Thus, reduced saturation can be indicative of cancer (although other conditions can also, more rarely, produce necrosis), but good saturation is *not* a reliable indicator of benign conditions. Artificial-intelligence pattern-recognition (AI-PR) methods are well suited for spectral classification when model-based analysis is made difficult by the large number of necessary input parameters resulting from heterogeneity. Nonetheless, there certainly are some spectral indicators that are understood, as discussed below, and we have ensured that the input parameters (derived from the spectra) for our AI analyses contain that information.

Two different AI-PR methods of spectral classification have been employed to assess the degree of correlation between pathology and spectral pattern differences: artificial neural networks and hierarchical cluster analysis. Artificial neural networks (ANNs) were selected for study because of the expectation by our group and other researchers[4] that ANNs would prove to be a generally useful method of tissue spectral classification. ANNs (and other AI-PR methods) are

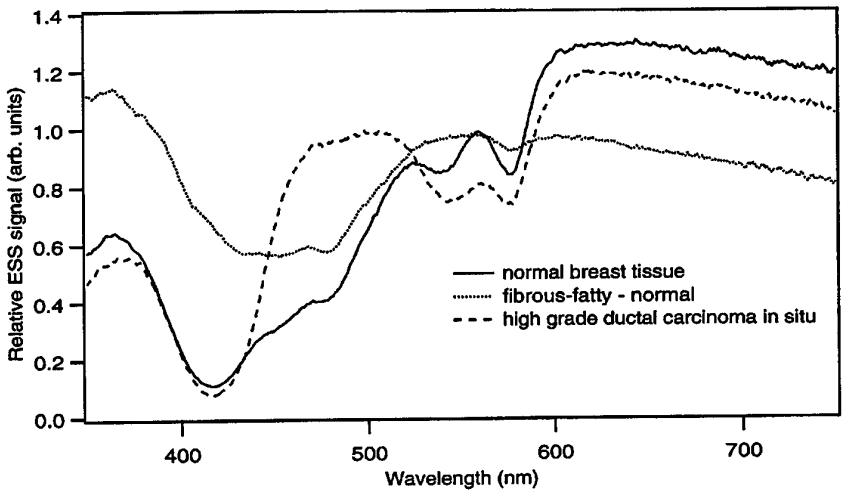

Fig. 3 Examples of ESS spectra for normal and malignant breast-tissue conditions.

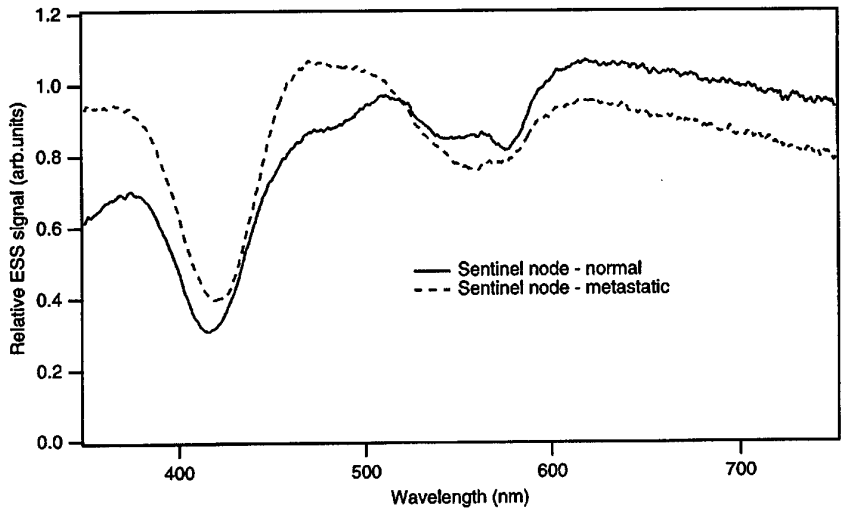

Fig. 4 Examples of ESS spectra for a reactive node, without tumor, and for node containing metastatic tumor.

known to be well suited for classification in systems where model-based classification is difficult.[18] As stated above, such is the case with ESS spectra of breast tissue because of its remarkable heterogeneity of tissue types. In light of this expectation, there was interest in our group to evaluate the relative merits of ANNs in ESS spectral analysis. Hierarchical cluster analysis (HCA) was also selected for study because it can serve as an alternative to the many approaches to classification that provide unbounded class regions (including ANNs, as well as linear discriminant analysis, regression analysis, etc.). The problem of unbounded class regions, and the benefits of using cluster analysis to avoid this problem, are discussed in detail by Osbourn et al.[19] In general, the problem with unbounded class regions is that a new sample will be classified in one of the available classes of a previously trained system, even if the input parameters of this new sample differ markedly from the other samples in the class to which it is assigned. This is not a problem when new samples are expected to be reasonably similar to previous samples. With optical biopsies, however, a number of variables can significantly alter the measured spectra. In addition to tissue heterogeneity, and even with relatively homogeneous tissue types, there can be experimental factors such as the presence of fluid (especially blood) trapped under the probe tip, or simply as a consequence of improper probe contact with tissue. It would thus be beneficial for a classification technique to be able to recognize samples that may have been adversely affected by such variables (i.e., "outliers:" samples that differ markedly from previous samples). Having bounded class regions, such as those produced through HCA, provides this ability to reject outliers. A more detailed description and comparison of these artificial intelligence methods for classification of tissue spectra for several different organ areas is the subject of a future publication currently in preparation.

Although both ANN and HCA methods are intrinsically statistical-based classification methods, some preprocessing or weighting of input parameters can be implemented to "assist" classification when some things about the underlying tissue optical properties are known. (Such preprocessing can, in effect, combine benefits of both statistical-based analysis and model-based analysis.) In its simplest form this means that the input parameters for training the AI methods, which are derived from the raw spectra, should be structured so as to include the spectral information known to have diagnostic relevance. For example, the presence of adipose (fatty) tissue in the measured volume is much more frequently associated with normal or benign breast conditions (for a given biopsy site) than with adenocarcinoma. Especially in postmenopausal women, and in mixed tissues, a developing adenocarcinoma will often "push" the fat out of the tissue volume where growth is occurring. Since adipose tissue has the characteristic absorption feature of beta carotene, the spectral bands containing that feature can be accounted for. (The absorption feature of beta carotene (450–500 nm) can be seen in both the fibro-fatty and normal spectra in Figure 3.) Another example is the mean redox state of the hemoglobin in the tissue. Hemoglobin desaturation is generally indicative of necrotic/malignant lesions, although the obverse is not true, and hypervascularization more frequently accompanies malignant tissue than normal or benign conditions. In short, it is valuable to include enough detail of the beta carotene and hemoglobin bands among the AI-PR input parameters. Finally, broad (large-spectral-range) slope changes are expected for malignant conditions in glandular tissue due to enlarged and denser nuclei, and other scattering differences are expected among tissue types.

For the data analyses presented here, some model-based preprocessing was implemented to ensure sensitivity to known factors, but no weighting of any specific parameters was implemented at this stage. This and other methods of data preprocessing can improve the classification accuracy, and will be treated more extensively in a future publication.

5 Data Processing and Analysis Results

For all analyses reported here the spectra from breast tissues and sentinel nodes were analyzed separately since they are

fundamentally different classes of tissue. All breast-tissue spectra were preprocessed by first normalizing each spectrum to the same total integral over the spectral range of 330–750 nm. Sentinel-node spectra were normalized for the range 330–590 nm. (See discussion below.) Thus, only spectral shapes were compared. These normalized spectra comprise information on both the spectral dependence of scattering parameters and absorption bands, but not total scattering efficiencies. Such data treatment eliminates concerns about differences in optical coupling and/or transmission among the several fiber probes used, although any potentially useful information in the total scattering efficiency is, admittedly, also lost. In the case of sentinel nodes, many exhibited strong absorption due to the presence of methylene blue dye (Patent Blue™), which had been used to assist the surgeon in locating the node during surgery. Therefore, sentinel-node spectra were also treated to subtract the feature of that absorption band, and were analyzed for the range of 330–590 nm.

In presenting the statistical results, sensitivity and specificity are defined in the standard way

$$\text{Sensitivity} \equiv \text{SE} = \text{TP}/(\text{TP}+\text{FN}),$$

$$\text{Specificity} \equiv \text{SP} = \text{TN}/(\text{TN}+\text{FP}),$$

where TP, FP, TN, and FN represent the numbers of true positives, false positives, true negatives, and false negatives, respectively, as determined by the corresponding histopathology.

5.1 ANN Classification

For ANN classification the normalized spectra were divided into 21 wavelength bands of 20 nm width (330–750 nm), with some of the intervals being smaller for sentinel-node spectra because of the reduced total spectral range (330–590 nm), and an average spectral intensity for each interval was calculated. For classification of breast-tissue spectra we also included slopes of the intervals as input parameters, and although more sophisticated ANNs can handle a large number of input nodes, ours was limited to about 20. Therefore, for breast-tissue spectra, principal component analysis was performed on the interval intensities to establish a smaller number of input parameters.[20] The resulting smaller number (4–6) of principal components based on spectral intensities were combined with a number of slopes for various spectral intervals, for a total of 12–16 input parameters.

With ANN classification a variety of network designs and transfer functions between neuron layers are possible.[21] The analyses presented here used a relatively simple flat, three-layer network, with input and output layers and one hidden layer of neural nodes, or "neurodes." As mentioned above, the input layer had 12–16 input parameters (neurodes) and the output layer always had two parameters, for malignant and nonmalignant conditions. The choice for the number of hidden-layer neurodes is somewhat arbitrary, and a typical starting point is to set it at half the total of input and output parameters, although sometimes a somewhat larger number is chosen if training is too slow. The transfer function (between layers) chosen for these analyses is a unipolar sigmoid (from 0 to 1), which is often used for parameters that can have smoothly varying values.[21] Network training was accomplished by "error back propagation," wherein the correct diagnoses (from histopathology) are presented at the output layer, and the network is run (transfer functions are varied) to minimize the error between the classified output and the known parameter. Eighty percent of the data samples where selected randomly for training and the remaining 20% were used for testing. This was repeated five times with disjoint testing sets, each with a different 20% of the samples, so that all of the data samples were used for testing, but none were used for both training and testing in the same run. Statistics were derived from the average of all five runs. Given the modest size of the data set, better statistics might have been achieved by the "leave-one-out" method where only one data sample is used for testing (and $n-1$ are used for training) and repeating the process n times, but this would have taken prohibitively long computer time.

5.2 HCA Classification

For HCA classification of breast-tissue spectra, the spectra were again divided into 20 nm intervals, and a preliminary cluster-template analysis was performed, as described below, in order to pre-select a subset of interval intensities to be used for input parameters. Similarly, preliminary template analysis was also performed with interval slopes in order to pre-select a reduced number of slopes as input parameters. A combination of these pre-selected intensities and slopes was then used as the set of input parameters for the full HCA analysis. For sentinel-node spectra only the interval intensities were used as input parameters, without any pre-selection.

With the hierarchical cluster analysis method the "training" consists of trying all possible different combinations of the input parameters, using multidimensional pattern recognition schemes to find clusters with no *a priori* input of actual classifications, and then comparing the resulting clusters with the known classifications of their members. Different criteria are possible for determining the multidimensional Euclidian "distance" between a sample point and a given cluster: nearest neighbor, farthest neighbor, or average distance.[20] For the analyses presented we chose the average-distance method. The combination of input parameters that yields the clusters of samples that best agree with each other, by having the same histology classifications, is the one that is chosen. For a given number of input parameters HCA generally requires more data than ANNs for statistical stability of the clusters (outputs). However, training can be faster than for ANNs. Once the input parameters for the best clustering are established, a new data sample can be tested for those parameters, to see which (if any) cluster it falls into. With our modest-sized data sets, and with the multiple input parameters required, the clusters found are sensitive to the specific choice of training set. Therefore, instead of an 80/20 split of the data, we applied the leave-one-out method, as described above, and the statistics are determined from the sum of all of the leave-one-out tests.

The results of data analysis are summarized in Tables 2 and 3. It can be seen that with the HCA method a few samples appear as unclassifiable, rather than as false positives or false negatives. It is especially interesting that, upon examination of the individual unclassified samples, about half of these outliers corresponded to samples that were classified incorrectly by ANN. The resulting sensitivities and specificities were

Table 2 Classifications for breast-tissue spectra.

	ANN	HCA
Number of specimens showing cancer histologically	13	13
Number of cancers optically diagnosed as cancer (true positives)	9	8
Number of cancers optically diagnosed as normal (false negatives)	4	4
Number of cancers with an indeterminate optical diagnosis (HCA only)		1
Sensitivity (%)	69%	67%
Number of specimens found normal histologically	59	59
Number of normals optically diagnosed as normal (true negatives)	50	44
Number of normals optically diagnosed as cancer (false positives)	9	12
Number of normals with an indeterminate optical diagnosis (HCA only)		3
Specificity (%)	85%	79%
% Classified (HCA only)		91.5%

Table 3 Classifications for sentinel-node spectra.

	ANN	HCA
Number of specimens showing cancer histologically	12	12
Number of cancers optically diagnosed as cancer (true positives)	7	10
Number of cancers optically diagnosed as normal (false negatives)	5	1
Number of cancers with an indeterminate optical diagnosis (HCA only)		1
Sensitivity (%)	58%	91%
Number of specimens found normal histologically	42	42
Number of normals optically diagnosed as normal (true negatives)	39	26
Number of normals optically diagnosed as cancer (false positives)	3	8
Number of normals with an indeterminate optical diagnosis (HCA only)		8
Specificity (%)	93%	76.5%
% Classified (HCA only)		83.3%

comparable for breast tissue, but for sentinel nodes HCA yielded significantly better sensitivity (91%) than ANN (58%), albeit at the cost of somewhat reduced specificity. It is our expectation that with adequately large data sets HCA with properly chosen input parameters will generally yield similar or better sensitivity and specificity, as compared with ANNs, but with HCA having the advantage of identifying ouliers as difficult to classify, rather than blindly forcing a classification. We believe this is a more realistic approach for clinical application of any new diagnostic method, and is comparable to other clinical measurement situations: for example, an invalid EEG measurement resulting when an electrode has poor contact. As expected with data sets for which there were significantly more negatives than positives but comparable accuracy, the negative predictive values (NPV) (87%–96%) were significantly higher than the positive predictive values (PPV) (40%–75%), where $NPV \equiv TN/(TN+FN)$, and $PPV \equiv TP/(TP+FP)$.

6 Discussion and Problems Encountered

It is not the purpose of this paper to present a detailed discussion of the structures and functions of different artificial-intelligence, pattern-recognition schemes for classification of tissue spectra, but rather to present the results of their implementation, with minimal refinement, for these preliminary data of our breast-cancer study. Although our ANN was limited to about 20 input parameters, with some ANN codes it is possible to have as many as 250 input parameters, which would have permitted us to present every spectral resolution element, for each spectrum, to a separate input node. This would have permitted capturing all spectral features. Also, a variety of other improvements and refinements are possible, including variations in the design of the ANN, and more sophisticated data preprocessing and choice of input parameters for HCA. A careful discussion of the relative merits of AI methods for tissue-spectra classification will require a full separate publication, which (as mentioned above) is in preparation. Also, with HCA robust clustering requires that the number of samples be much greater than the number of input parameters. For the sizes of our data sets this condition was marginally met, and we expect significant improvement in the statistics as data sets grow. Nonetheless, we believe these preliminary results are compelling enough to merit presentation to the scientific community.

In actual clinical implementation of the ESS instrumentation, it became evident that some attention must be given to avoiding excessive amounts of blood on the tip of the fiber probe, as this can obscure information about the amount of tissue perfusion, or even block much of the scattering spectral information (due to the strong absorption by hemoglobin). Thus, it was sometimes necessary to sponge, or rinse with saline, the surgical resection surface to be measured, and/or to wipe the tip of the probe. Also, for sentinel nodes the accuracy in co-registration between optical and histology sites left some uncertainty when only a small region of the node exhib-

ited metastasis. Improvements are planned for the co-registration between optical measurement and histology for sentinel nodes.

Another problem encountered on a few occasions was that the ambient lighting of the surgical suite (with the high-intensity directed-beam lighting typically found in theater) would occasionally cause too much scatter through tissue into the collection fiber of the probe, using up much of the detector's dynamic range, and making it difficult for the system to perform an accurate background subtraction. This was remedied by either having the surgeon momentarily shadow the strong light from the measurement site, or by a redesign of the probe fixture to incorporate a small shadow mask. (Of course, the strong lights could be temporarily aimed away from the surgery or reduced in intensity while the ESS measurements are being made, but we wished to minimize any inconvenience to the surgical team.)

Finally, in the development of any new diagnostic technique, the accuracy and robustness of the "gold" standard, histopathology, are always a concern. In our case all pathology reports were provided by a specialist breast pathologist (S.L.) of the University College London Hospitals. (For the purposes of these analyses, ductal carcinoma *in situ* (DCIS) was always classified as cancer, since the treatment consequences are the same regardless of whether the DCIS already shows signs of invasiveness or not.) Careful attention has been paid to adequacy of tissue samples, correlation with optical measurement sites, and consistency of pathology reporting terminology, although further improvements are planned. As the program enlarges, and incorporates other medical centers, agreement among pathologists will be addressed, and slides will be read by multiple pathologists. Methods will be assessed to resolve conflicting reports. Nonetheless, the reliability of histopathology being less than 100%, this type of correlation is fundamentally limited in accuracy, and determination of the ultimate capability of the new method requires long-term outcome studies.

7 Conclusions

We have described a research program designed to test the value of elastic-scattering spectroscopy as a real-time diagnostic tool and as a diagnostic aid to surgical/therapeutic procedures for breast cancer. We have presented, as preliminary data, the results from the first 31 patients of a larger program. The modest sizes of the data sets notwithstanding, the results of spectral classification by two different methods of "artificial intelligence" pattern recognition show promise for good agreement with pathology. This allows us to be hopeful that as the data sets grow, we will be able to successfully test the predictive capabilities of already trained spectral classification schemes.

Acknowledgments

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LASER TREATMENT OF BREAST TUMOURS

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1. INTRODUCTION

There are around 570,000 new cases of breast cancer in the world each year with breast cancer being the leading cause of death in women [Cancer research campaign, 1996]. Breast cancer survival rates are extremely variable with a current 5-year survival in the UK of 68%. This compares poorly with the rest of Europe (average 73%) [Anonymous, 1999] and the US (average 84%) [Office for national statistics, 1999]. No other cancer produces such an emotive response amongst the general public, due mainly to increased public education and high profile coverage in the popular press. With ever increasing activity of patient advocate groups, attention has been re-focused on all aspects of breast cancer care, causing renewed efforts to improve prevention, detection and treatment. Despite many recent advances however, a universal cure for breast cancer is still some way off. Research is currently under way to see if laser treatment has a role to play in the treatment of breast cancer, but at present it can only be regarded as a research technique. Benign conditions of the breast attract much less media attention, but laser therapy is looking particularly promising for the treatment of fibroadenomas of the breast, a common condition that causes hard lumps to appear in the breasts of young women.

2. BREAST CANCER

2.1 SURGERY

Little has really changed over the past century in the surgical treatment of breast cancer in terms of technology. Most surgeons still perform the operation with a scalpel, some technique like cautery to control bleeding and sutures. What has changed is a move towards more conservative, breast preserving surgery. This shift has only really taken place in the past 20 years. This has followed the publication of several large studies which evaluated the long term survival after localised surgery in conjunction with radiotherapy compared with traditional mastectomy alone [Fisher et al., 1995;Jacobson et al., 1995;Veronesi et al., 1994]. Breast conservation surgery generally means the excision of the cancer with a surrounding rim of normal tissue. Most would accept that a 1cm margin around the tumour is adequate although there is still much debate about this. Although local recurrence rates are higher with lumpectomy compared to wide local excision, the overall survival remains the same [Veronesi et al., 1994]. There is a trade off between removing enough tissue to keep local recurrence to a minimum against removing too much, with a resultant poor cosmetic outcome.

Lesion size is the main predictor of whether breast conservation surgery is possible. An arbitrary cut off figure of less than 4cm is often quoted but it is highly dependent on the relative size of the breast and the position of the lesion i.e. excision of a moderate sized tumour in a small breast may have a worse outcome than a larger lesion in a large breast. Also, excision of tumours in the lower aspect of the breast often has a poorer cosmetic outcome compared to those in the upper part due to a pulling down of the nipple through removal of supporting tissue and contraction of the scar.

What is evident from these trials is that surgery to remove the primary breast tumour serves only to achieve local control of disease and may have little effect on the overall outcome. Any technique that can destroy tumour tissue in the breast just as well without the need for surgical excision has obvious attractions.

2.2. THERMAL ABLATION

The use of heat to treat breast cancer has been documented as far back as 3000-2500 BC with mention of "cauterization with a fire stick" [Breasted, 1930]. As heat has the obvious ability to destroy tissue, early physicians saw its potential to destroy cancerous lesions, with the added benefit of achieving cautery to blood vessels at the same time. Thermal destruction of tissue can occur in two ways. Traditionally, any thermal ablative technique has relied on explosive vaporisation (true ablation) by using a high power energy source to cause intracellular water to reach 100°C and therefore vaporise, causing rupture of the cell membrane. The second method is by protein denaturing (thermal coagulation) which utilises a lower power source to raise the temperature above 50°C for a set period of time. It has been shown that temperatures as low as 41-42°C will cause cell death if exposure is prolonged [Overgaard and Suit, 1979], but as the temperature is increased the time to achieve this decreases [Borrelli et al., 1990]. As a rule of thumb, the time required to kill tissue halves for each 1^0C increase in temperature. The mechanism of tissue destruction is complex but most probably is related to reduced pH, hypoglycaemia, vasoconstriction, increased capillary permeability and oedema [Vaupel et al., 1988]. Temperature changes in the opposite direction are also effective. Extreme cold has been used for many years as a method for destroying tissue [Gage, 1998]. Indeed 'frostbite' can be just as damaging to tissue, if not more so, than a burn!

As the precision and sophistication of energy delivery devices has evolved, so interest in interstitial thermal therapy has increased. This is the direct delivery of heat into lesions in the centre of solid organs, minimising the effects in the overlying normal tissues. The rate of heat delivery is much slower than with techniques for immediate vaporisation of tissue, to avoid explosive effects deep within a solid organ, but the precision is usually much better. This is the "gentle casserole" rather than the "flame grilled" effect. Such methods are gaining interest for the treatment of a variety of benign and malignant tumours where evaporative ablation is undesirable. All interstitial therapy is critically dependent on imaging to define the limits of the lesion to be treated, to insert the energy delivering device and to monitor the efficacy of treatment. Appropriate imaging techniques are evolving rapidly [Lamb and Gedroyc, 1997] and are discussed later in this chapter.

2.3.1. THERMAL ABLATIVE TECHNIQUES

There are five main methods of tissue ablation by changing the tissue temperature, all of which can be adapted for minimally invasive use. Three of these, laser photocoagulation, radiofrequency heating and microwaves, utilise electromagnetic radiation. The other two are ultrasound (pressure waves) and cryotherapy (direct damage by freezing). For interstitial use, laser and radiofrequency heating are the most convenient as the probes can be inserted into tissue through thin needles. To some extent this is also true for microwaves, but cryotherapy requires a wide enough probe to permit circulation of the coolant, such as liquid nitrogen. Therapeutic ultrasound is usually delivered via phased arrays of transducers so it is not necessary to insert anything into the tissue, but this has the limitation that it is difficult to produce effects accurately more than a few cms deep in tissue or if there are extra tissue planes through which the beam has to pass before reaching the target lesion. The discussion in this chapter will be limited to Interstitial Laser Photocoagulation (ILP).

2.3.2. Interstitial Laser Photocoagulation (ILP)

This procedure was first described in 1983. [Bown, 1983]. The concept is simply that a laser fibre (typically 0.2-.0.4mm core diameter) is inserted directly into the target tissue (usually through a needle of about 18 gauge) so light is delivered to the tissue from the end of the fibre as a point source (bare tip fibre) or emitted from the end section of the fibre (diffuser fibre, where the diffuser section can be up to several cm long). The energy is absorbed within the surrounding tissues causing local thermal necrosis. One or more fibres can be used [Steger et al., 1992]. Various lasers have been tried but the most convenient are either a semiconductor diode or a Nd:YAG laser, at wavelengths between 805 and 1064nm. The technique is sometimes referred to as Laser Interstitial Thermal therapy (LITT) or Interstitial Laser Thermal Therapy (ILTT).

For treatment of tumours of the breast, the procedure can be carried out under local anaesthetic and mild sedation. Up to 4 needles can be placed directly into the tumour either under ultrasound or MR guidance (Fig 1). Optical fibres are then inserted through each needle. Once the fibres are in place the needle is withdrawn slightly so the bare ends of the fibres lie within the tumour. Previous work has demonstrated that the size of the laser induced necrosis is more predictable when the bare end of the fibre is pre-charred before insertion [Amin et al., 1993a;Harries et al., 1994]. This leaves a deposit of carbon particles on the tip of the fibre, which absorb the laser energy, so making the fibre tip a point source of heat. Without this pre-charring, some patients have shown no discernible treatment effect, probably because the available energy is absorbed in a larger volume of tissue so there is not enough energy per unit volume of tissue to produce a biological effect. The volume of tissue treated from each fibre position can be increased by using diffuser fibres instead of bare tipped fibres, although these are usually larger in diameter than the bare tipped ones, so require larger needles for insertion. This causes more trauma to the overlying tissue through which the needle has to pass to reach the target lesion. Diffuser fibres are also relatively difficult to manufacture to tolerate the high temperatures generated during light delivery. Once the fibres are safely secured in position in the target tissue, the laser is activated, typically for 10 minutes at 2-3 watts per fibre. This gives a characteristic zone of tissue destruction (necrosis) with central charring (sometimes with liquefaction), a surrounding area of heat fixed tissue consistent with coagulative necrosis and an outer haemorrhagic rim. These changes are seen in surgical specimens removed a few days after ILP.

Microscopically, the central area contains carbonised and necrotic debris. Within the surrounding heat fixed zone, the features are of morphologically normal cells but with featureless, heavily stained, nuclei consistent with coagulative necrosis (Fig 2). Within the haemorrhagic peripheral zone the cells are less severely disrupted, with the only indication of cell damage being some increased staining in the nuclei. Also within this zone are proliferating fibroblasts (cells that lay down scar tissue) and damaged blood vessels with extravasation of red blood cells. It has been shown using immunohistochemical staining (NADH-diaphorase) that all these areas contain dead tissue [Mumtaz et al., 1996]. These findings have been confirmed recently by a group in Stanford who are evaluating radiofrequency ablation of breast tumours [Jeffrey et al., 1999].

2.3.3. Clinical Results

The earliest reported case of a laser treated breast cancer was by Steger et al in 1989 [Steger et al., 1989]. Since then there have been several reports of studies investigating the feasibility of treating breast cancers in this way [Akimov et al., 1998] [Mumtaz et al., 1996] [Harries et al.,

1994] [Dowlatshahi et al., 2000]. In all cases, the laser treatment was either purely a research procedure (in which case, ILP was followed by surgical removal of the treated cancer for microscopic examination of the treated tissue) or was a palliative procedure undertaken when other treatments such as surgery or radiotherapy had failed or the patient had refused alternative treatments.

Harries et al [Harries et al., 1994] treated 44 patients with breast cancer prior to routine surgical excision. Predictable areas of tumour necrosis (median diameter 14mm) were achieved using a pre-charred fibre. Only one minor complication occurred which was a small bleed from the puncture site. Mumtaz et al [Mumtaz et al., 1996] reported the outcomes of a further 20 patients with similar results but with more sophisticated imaging, as discussed below.

More recently, a group in the Ukraine [Akimov et al., 1998] presented their results in the treatment of 35 breast cancer patients. They used a Nd:YAG laser at slightly higher power outputs (2.5-6 W). Twenty-eight patients underwent laser therapy prior to surgery and a further 7 had ILP as their only invasive treatment. All tumours again showed laser induced necrosis with increasing diameter of destruction with increasing power and energy levels. There were slightly more complications, however, with 4 minor skin burns and one gaseous rupture. These were probably related to the increased power employed. As the first two studies have shown, 2.5 Watts is adequate to produce cell death without the fear of evaporative ablation. Of the 7 patients who did not undergo surgery, 3 were post-menopausal with severe co-existing medical disease. Following ILP they all showed a good response to treatment with tumour shrinkage A further 3 patients with metastases at the time of diagnosis underwent palliative ILP. Two of the three showed a reasonable local response to treatment. The third however showed no change in tumour size. The final patient was a young woman who refused surgery. Unfortunately she progressed quickly with aggressive metastatic disease. Overall, however, their results were again encouraging with few complications.

The most recent study by Dowlatshahi et al [Dowlatshahi et al., 2000], reported the results of 36 patients with tumours less than 2cm in diameter treated with an 805nm Diode laser. They employed a single water-cooled diffusing fibre that allows light to spread from the tip equally in all directions and also maintains the temperature below 100^0C, to avoid charring, although this type of fibre is considerably larger in diameter than those used in the earlier studies. All patients underwent surgery 8 weeks later. Examination of the excised tissue showed that there was complete tumour ablation in 66% of cases. Only 2 minor skin burns were encountered with no serious complications.

The only other report of thermal ablation of human breast tumours in-vivo was by Jeffrey and co-workers who described the effects of radiofrequency ablation prior to routine surgery in 5 patients [Jeffrey et al., 1999]. Using a single 15-gauge electrode they demonstrated partial tumour ablation in all patients with no complications. The aim was to assess the feasibility of destroying breast tumours by this method and they intentionally left part of the tumour untreated to evaluate the margin between ablated and non-ablated tissue. The size of necrosis was similar to laser therapy although the treatment times were much longer, 30 minutes versus an average of 10 minutes for ILP.

These studies have demonstrated the feasibility of producing local destruction of breast cancers using thermal ablative techniques. None of the studies set out to completely destroy breast cancers, although this was achieved in some cases.

Having shown that interstitial therapy can ablate cancerous tissue in the breast the next major challenges for the clinician are to produce a non-invasive means of assessing in-vivo whether the cancer ablation is complete or whether viable malignant tissue has been left behind and to be sure that laser treated areas heal safely. These aspects are addressed in the following sections of this chapter.

2.3.4. Imaging for ILP of breast cancer

The main goal with any thermal treatment is to achieve complete local destruction of tumours, with minimal damage to surrounding normal tissues and fewer complications than after conventional surgery. One of the key factors is the ability to assess the extent of tumour destruction *in-vivo* accurately, either at the time of treatment or shortly afterwards. The gold standard for assessing completeness of tumour clearance is microscopic examination of surgically excised tissue to show that there is no residual cancer at the resection margins. The challenge for those advocating interstitial therapy is to find an equally reliable way of detecting residual viable cancer after treatment without having to remove the treated tissue from the patient. An ideal imaging modality would provide real-time assessment of the extent of tissue destruction. This would allow adjustment of treatment parameters (laser power and treatment time) or re-positioning of probes/fibres during treatment to ensure complete ablation of the tumour, with a 'safe' surrounding rim of normal tissue. Alternatively, it may be easier to look some time after treatment to detect tumour persisting or recurring in the treated area. Four imaging modalities are being evaluated for assessing the immediate and longer term efficacy of ILP: ultrasound scanning (US), CT scanning (computerised tomography), MRI (magnetic resonance imaging) and PET (positron emission tomography).

US is the simplest option and was tried in the early work with ILP on the liver and breast [Amin et al., 1993b;Harries et al., 1994;Steger et al., 1989]. Steger found US useful for placing the needles and fibres prior to treatment and some changes could be detected during energy delivery. However, US images did not give an accurate indication of the extent of thermal damage either at the time of treatment or subsequently. The studies by Amin and Harries reported similar findings. In the more recent study by Dowlatshahi, it was suggested that by using an intravenous contrast agent (Optison), US could detect the loss of blood flow in coagulated tissue [Dowlatshahi et al., 2000], although there is some doubt about how accurately the boundaries of the necrosed tissue could be defined as the ultrasound results were not correlated with histological findings. The same study presented data on the use of PET scanning, pre- and post- laser treatment. They found reasonable correlation between isotope uptake and residual tumour but this was only done on 4 patients as it is an expensive and time consuming option.

Before the widespread availability of MRI, dynamic enhanced CT was investigated for determining the extent of tissue destruction and residual tumour after ILP [Harries et al., 1994], [Amin et al., 1993b]. Characteristically the necrotic tissue appeared as avascular areas (no uptake of contrast material injected intravenously). This was confirmed by core-cut biopsies, which did not find any residual viable tumour in these areas. In the paper by Harries, 6 patients had CT with subsequent histopathological correlation on the resected specimen. This showed reasonable

accuracy for detecting the volume of treated tissue. There have, however, been no larger trials evaluating CT in the breast. CT is not a conventional imaging modality for breast cancer and it exposes patients to ionising radiation, which ultrasound and MRI do not, so it is unlikely to become the investigation of choice for this indication. CT has proved much more effective for assessing the results of ILP on liver tumours [Amin et al., 1993b].

It now appears that MRI has the best chance of achieving the main goals required for monitoring breast cancer ILP. This applies both for real time monitoring to see the extent of tissue damage as the laser is firing and for follow up imaging to look for persistent or recurrent cancer. Thermally destroyed tumours show signal change on both T1 and T2 weighted MR sequences [Klotz et al., 1997;Solbiati et al., 1997]. For breast MRI other than at the actual time of light delivery, an intravascular paramagnetic 'enhancement' agent (usually containing gadolinium) is used. This concentrates preferentially within breast tumours (most likely in regions of angiogenesis, the new blood vessels that develop in the regions where cancers are spreading into adjacent normal tissues) and therefore appears as a bright area on the image (increased signal intensity). Characteristically, thermally coagulated tissue loses this enhancement whereas in residual viable tumour, the increased signal intensity is maintained. The use of dynamic fast sequence scanning (taking repeated scans at intervals of a minute or two after injection of the contrast agent) allows the enhancement characteristics of specified areas to be studied. There has been much debate about the ability of variations in signal intensity with time to differentiate between benign and malignant enhancing lesions, but for follow up after ILP, the most important aspect is to see if any of the tumour enhancement that was present prior to ILP remains after treatment. Care must be taken when interpreting the images as in the first month or so after treatment, there is an inflammatory reaction in the surrounding tissue at the edges of the ILP treated zone, which may itself give rise to a zone of increased uptake of contrast agent (Fig 3). There is not yet enough data available to be able to judge whether dynamic enhancement characteristics will have any role to play in determining the nature of residual enhancement after ILP [Mumtaz et al., 1996].

There have been a multitude of papers in the recent literature exploring the possibilities of using MR to guide, monitor and follow up thermally ablated tumours, although only one has specifically assessed its accuracy following ILP in breast cancer [Mumtaz et al., 1996]. Pre- and post- treatment scans were performed and compared to the pathological specimen following routine surgical excision a few days after ILP. Imaging immediately after ILP did not show good correlation with the final histology, whereas delayed imaging (more than 24 hours after ILP), was much more accurate (Fig 4).

Contrast enhanced imaging gives an accurate picture of viable cancer in the breast, but it cannot be used to follow real time changes during light delivery as the time taken for the contrast agent to clear after one injection is comparable to the time required for laser light delivery, so it is only possible to get an image of the situation at one point in time. Further, it has been well documented that contrast enhanced scans taken within a few hours of ILP do not give a true picture of the extent of tumour destruction, as this takes a day or two to evolve. However, there is another option as MRI can be used without administration of a contrast agent for real time temperature mapping during treatment. The MR parameters that vary with temperature are signal intensity on T1 weighted images [Cline et al., 1994], signal intensity change due to diffusion [Bleier et al., 1991] and signal phase change due to proton frequency shift [de Poorter et al.,

1995]. Most investigators have concentrated on T1 signal intensity change and Proton Resonance Frequency Shift (PRFS). Both have their relative merits but also certain disadvantages.

With increasing temperature, the T1 relaxation time lengthens, so signal intensity decreases. This occurs in a relatively linear fashion until the temperature reaches around 60^0C when a plateau is reached [Lamb and Gedroyc, 1997]. Acquisition times are fast (around 3 seconds per slice) and thermal colour coded images can be obtained (Thermo-TurboFLASH, Siemens). The main disadvantage with this method is the antagonistic decrease in T1 relaxation time within coagulated tissue. This fact has lead some investigators to concentrate on frequency shift imaging as this is dependent on temperature change only [Graham et al., 1999]. PRFS has also been shown to be more accurate than signal intensity change for temperature mapping [Moriarty et al., 1998]. The main drawbacks are slow acquisition times (up to 1 minute per slice) and marked sensitivity to motion artefacts. These problems can be partly overcome with faster imaging (spoiled gradient echo – 20 seconds per slice) and immobilisation of the tissue in question. The need for sub 20 second imaging is also questionable as most coagulation methods have treatment times extending over several minutes. Of greater concern, however, is the difficulty of using this technique in tissues (like the breast), which contain fat, as this causes temperature-induced susceptibility changes [de Poorter et al., 1995]. This makes signal intensity changes more suitable for monitoring thermal ablative methods within the breast.

Careful studies in the normal rat liver have shown good correlation between real time MR images and the extent of ILP induced thermal necrosis [Roberts et al., 1997a]. Fig 5, from this report, shows the MRI changes at the end of a 500 sec treatment and an image taken 360 sec after the laser was turned off, when the tissue temperature had returned to normal. A subsequent clinical paper from the same group reported real time MR images of ILP being applied to liver metastases and showed that the extent of laser induced necrosis correlated well with contrast enhanced CT scans taken a few days later [Roberts et al., 1997b].

Real time, dynamic imaging (looking at the temperature changes) is the ideal option for monitoring ILP in the breast as the images can be compared with contrast enhanced images taken prior to ILP. However, much research remains to be done to establish what changes on the scans really mean in terms of whether the tissue being imaged is viable or not. The MR demonstrates changes due to the increase in tissue temperature and the consequent biological changes, but it is difficult to be sure how severe these changes need to be to indicate a loss of tissue viability, which depends on both the temperature reached and the time for which that temperature increase is maintained. The ultimate aim is to continue energy delivery until the extent of thermal tissue destruction covers the entire tumour volume, together with a surrounding rim of normal tissue (in an attempt to ablate nests of cancer cells that may have spread beyond the cancer volume detected on the scans). This is still some way in the future and requires further developments in MR scanners. Adjusting the position of laser fibres during ILP requires an open magnet scanner (to give easy access to the tissue being treated), but current open magnets only have magnetic fields up to 0.5 Tesla, which is not really high enough. Good dynamic imaging of temperature changes requires more like 1.5 Tesla, which is currently only available in larger, closed scanners, although this is likely to change in the relatively near future.

Thus contrast enhanced MRI is the most accurate technique for documenting the extent of thermal necrosis in the tumour, but cannot give answers at the time of treatment. If residual

cancer is found some time after ILP, treatment would have to be repeated. ILP does have the major attraction that, unlike radiotherapy, it can be repeated at the same site, several times, if necessary.

3. Healing of breast tissue after ILP and treatment of benign fibroadenomas.

Until it is possible to tell with confidence from MRI or some other form of non-invasive imaging whether laser ablation of a cancer in the breast is complete or not, it would be unethical not to follow laser treatment by surgical excision. This makes it impossible in the current state of knowledge to follow how laser treated breast cancers heals. An ethical way around this dilemma is to treat benign lesions in the breast and follow how the zone of thermal necrosis heals as the body's response to any dead tissue in the breast, cancer or otherwise, is likely to be the same. By serendipity, this approach has led to the realisation that ILP is an attractive option for treating fibroadenomas.

Benign fibroadenomas have probably caused more unnecessary anguish to young women than any other breast problem. They are by far the most common cause of a discrete breast lump in young women and reach a peak incidence between the ages of 20 and 30 [Dixon et al., 1996] although they are not an uncommon finding in older women. They are often only discovered through routine screening mammography, where they appear as well circumscribed lesions, often with characteristic "popcorn calcification". Originally classified as a true benign tumour, they are now regarded as an aberration in the normal changes that occur in the breast throughout life. This has lead to introduction of the term ANDI (aberrations of normal development and involution). Much debate has arisen in recent years about the natural history of fibroadenomas, raising the question of which ones should be treated. In simple terms, around a third will get larger, a third smaller and a third will remain the same size [Carty et al., 1995;Dent and Cant, 1989;Wilkinson et al., 1989]. There is no evidence that they ever turn into cancer, although the two diseases can co-exist.

It can be argued that all but the most rapidly growing fibroadenomas should receive a trial of conservative management (ie no active treatment), and this is becoming normal practice in the UK. There is, however, a sub-group of women who request early excision due to fear of leaving a lump in-situ or of cancer being missed. Others will request surgical treatment at some point down the line, should their lump fail to resolve spontaneously. Few women would admit to being totally happy about leaving a fibroadenoma indefinitely, especially if it is particularly prominent.

As these lesions are benign, it is not critical that 100% of the lesion is treated as any residual fibroadenoma tissue does not pose a danger to the patient and can be treated again later if required. Further, the lesions have well defined margins, unlike cancers, from which nests of cancer cells may infiltrate the tissue surrounding the main bulk of the tumour. This makes imaging simpler, so ultrasound scanning, which is cheaper and more readily available than MRI can be used for guiding ILP and for follow up assessment (Fig 6). For larger fibroadenomas multiple fibres can be used (typically up to a maximum of 4). These are spaced as equally as possible within the tumour. It is important to check the position of each needle in two planes prior to light delivery as although a needle can appear central on one view, it can in fact be near the edge of the target lesion in another plane. For very large fibroadenomas, it is sometimes not

feasible to coagulate it all at one sitting. Areas of coagulated fibroadenoma can, however, be distinguished from non-treated areas on ultrasound scans (fig 7).

The original aim of treating fibroadenomas was to see how zones of thermally coagulated tissue in the breast healed and this has been assessed by serial US scans. These have shown the change in size of the lesions and the characteristics of laser coagulated tissue. Typically, ILP treated areas of fibroadenoma become hyperechoic (bright echo pattern) compared to the normal hypoechoic (dark echo) pattern (fig 7). After ILP, some of the smaller fibroadenomas can become quite indistinct and difficult to distinguish from the normal surrounding breast tissue, presumably because the area of coagulation extended into the surrounding normal tissue. Nevertheless, the remarkable finding has been that the coagulated tissue is gradually absorbed by the body's normal healing processes over a period of months. As long as the lump has been completely treated, in due course it disappears entirely, although this may take up to a year. Patients comment on some soreness in the treated area for a few days after treatment and the lump may initially swell (due to oedema) before shrinking, but the patients do not feel generally unwell in any way.

There have only been two published studies using ILP for fibroadenomas [Basu et al., 1999;Lai et al., 1999]. Lai et al treated 29 fibroadenomas with a mean diameter of 25mm (range 14-35mm). These patients were scheduled for conventional surgical excision, but were offered ILP as an alternative, on the understanding that they could still have surgery at a later date if they so wished. Twenty eight of the 29 lesions treated decreased in size on serial ultrasound. Only 6 patients continued with the original plan of surgery, and most of these chose surgery soon after ILP. It later became apparent that if they had waited longer, the fibroadenomas would probably have shrunk as a result of the ILP. The remainder were happy with the gradual reduction in size following ILP and declined further intervention. The average decreases in size documented were 38%, 60% and 92% at 3, 6 and 12 months respectively. 14 patients were followed up for one year, by which time none had a palpable lump and only one lesion was still detectable on ultrasound. There were no serious complications. Three patients had minor skin burns around the puncture site, either because the fibre slipped during treatment or the fibre had been positioned too superficially in the breast. This problem has been solved in more recent treatments by cooling the skin with iced water during light delivery.

Basu et al used a similar technique, the only real difference being the use of a Nd:YAG laser operating at 1064nm rather than the semi conductor diode laser (805nm) used by Lai et al. Twenty-seven patients were treated for lesions averaging 16mm in diameter, smaller than those in Lai's study. Follow up was shorter (8 weeks) but there was a larger average decrease in size at this time point (60%). Ten patients with residual lumps underwent excision biopsy. Again no serious complications were observed although 8 patients had minor skin burns around the puncture sites and two had a sterile, self limiting discharge.

These papers have demonstrated the safety and efficacy of ILP for treating fibroadenomas. The smaller number of skin burns in the first study is probably attributable to the use of an oblique course for needle placement when the lumps were superficial, rather than going directly through the skin overlying the lesion. Also cooling of the breast with cold saline may play a positive role in preventing this complication. Both studies demonstrated an increase in size over the first two weeks after ILP, presumably due to swelling from an inflammatory response, before a gradual

decrease in size over the following months. It is important to warn patients undergoing ILP that this is likely to happen.

4. ILP in other organs

Much of the early experimental work on ILP was done on normal animal livers, as the liver is such a forgiving organ and heals well after a localised thermal insult. In consequence, the first clinical studies were for the treatment of isolated metastatic tumours in the liver, most often in patients who had previously had cancers of the colon removed surgically [Steger et al., 1989]. Amin et al described their experience in the treatment of 55 liver tumours in 21 patients. 82% of patients fulfilled the UICC (Union Internationale Contre le Cancer) criteria for at least a partial response (>50% reduction in tumour volume) [Amin et al., 1993b]. Recent results have concentrated on the long-term outcome in over 500 treated patients with a median survival of 27 months and a 5-year survival rate of 26%. This is comparable with patient outcomes following operative treatment for liver metastases at the same institution, of 33 months median survival and 30% 5 year survival respectively [Dodd et al., 2000]. Another group reported a mean survival of 35 months [Vogl et al., 1997] after ILP for liver metastases.

Some work has been published on the treatment of benign prostatic hyperplasia. Here, the aim of treatment is to reduce the volume of the prostate gland to improve urinary flow [de la Rosette et al., 1997], although there is some doubt about whether ILP is really any better than the conventional surgical approach of shaving out the part of the prostate causing obstruction. Another promising clinical use is in the treatment of moderate sized uterine fibroids. These are benign tumours of the womb that can cause heavy and painful periods or infertility. Two groups have shown that ILP can shrink these lesions. Law et al demonstrated a mean reduction in tumour volume following ILP of 37.5% at 3 months in 12 female volunteers, although gave no indication of clinical benefit [Law et al., 1999]. Visvanathan et al treated 30 fibroids in 24 symptomatic patients and documented shrinkage in 23 lesions. [Visvanathan et al.2002]. Thirteen patients reported an improvement in symptoms of abdominal discomfort, urinary frequency or dysmenorrhea (painful periods) and one delivered a healthy child, having been unable to conceive prior to ILP. Yet another indication is for treating osteoid osteomas. These are benign but painful tumours of bone that often occur in sites that are very difficult to get to by conventional surgery, but where it is straightforward to insert one or more needles under CT guidance for ILP. ILP gives an almost immediate cure [Witt et al., 2000]. It has even been used for small but inoperable lung tumours [Brookes et al., 1997].

5. CONCLUSION

The idea of using ILP as an alternative to surgery for the destruction of small breast cancers is attractive, but it is important to keep a clear picture of the role it might play in the overall management of these patients. Local destruction of the cancer is only one part of the management of patients with this unpleasant disease and most will require additional treatment such as radiotherapy, hormones or chemotherapy, whether they have ILP or surgery. Further, it must be recognised that there is still a long way to go before there will be enough confidence that MR imaging (real time or delayed) can detect any viable cancer persisting after ILP, for surgeons

not to follow ILP by a conventional surgical excision. However, if this stage is reached, the benefits are the general simplicity and safety of the technique without the need for a general anaesthetic and the good cosmetic outcome (no scarring and minimal change in the shape and size of the breast). In an age where breast conserving surgery is standard practice, it could provide an alternative for some patients with breast cancer. There are particular attractions for patients whose general condition is poor, or who simply refuse surgery. For benign fibroadenomas, where precise imaging is not so vital, the evidence of efficacy and safety is now strong enough to offer ILP to all patients as an alternative to surgery.

6. Legend for figs:

Fig 1. Needles inserted into breast with fibres passed through needles into the tumour to be treated.

Fig 2. Photomicrograph of a breast cancer treated with ILP and removed by surgery a few days later. A small amount of charring is visible around the needle track (arrow) with coagulation of the cancer glands outside this zone.

Fig 3. Pre- and post laser contrast enhanced MRI scans of a small breast cancer. a) pre-treatment scan showing marked enhancement at the site of the cancer (arrow). b) scan 2 days after ILP showing loss of enhancement in the cancer with a new, fainter ring of enhancement around the treated area indicating inflammatory changes in the surrounding normal tissue as a result of the treatment.

Fig 4. Graph correlating the maximum diameter of remaining viable cancer after ILP as measured by contrast enhanced MRI and by conventional histology on the tumour removed surgically a few days after ILP (reproduced with permission from Mumtaz et al Radiology 1996 {Mumtaz, Hall-Craggs, et al. 1996 67 /id})

Fig 5. Representative images from dynamic FLASH monitoring of ILP to a normal rat liver. Under general anaesthesia, the abdomen of the rat was opened and a lobe of liver (straight arrow) gently eased out of the abdominal cavity onto the skin. a) baseline showing site of insertion of fibre (curved arrow). b) 500 sec after turning on laser to deliver 1W of laser light at 805nm through a bare tip fibre. c) 360 secs after laser had been turned off. The limits of the laser induced effects in b) and c) are shown with arrows and corresponded closely to subsequent microscopic examination of the treated tissue. (reproduced with permission from Roberts et al, Min Invas Ther & Allied Technol 1997 [Roberts et al., 1997a].)

Fig 6. Ultrasound scan showing a laser fibre (arrow) positioned in a fibroadenoma

Fig 7. Ultrasound scan to show a partly treated fibroadenoma. The central area (thick arrow) has been treated and has a bright echo pattern. The sections on either side (thin arrows) have not been treated and have very few echoes.

Fig 1

Fig 2

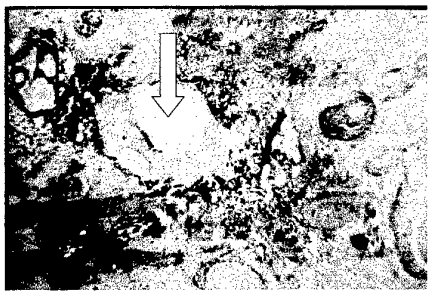


Fig 3a

Fig 3b

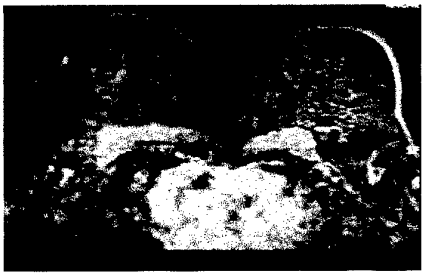


Fig 4

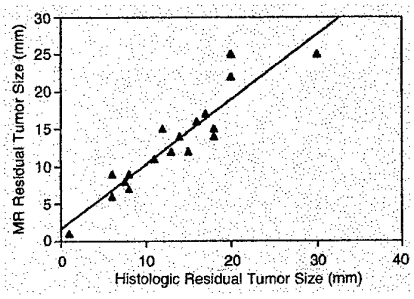


Fig 5a

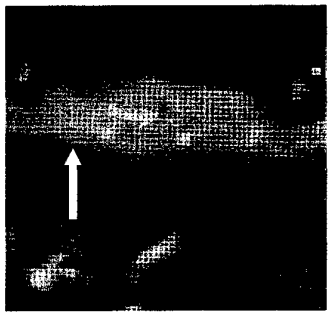


Fig 5b

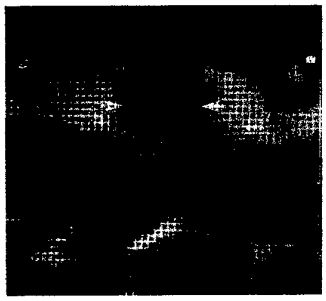


Fig 5c

Fig 6

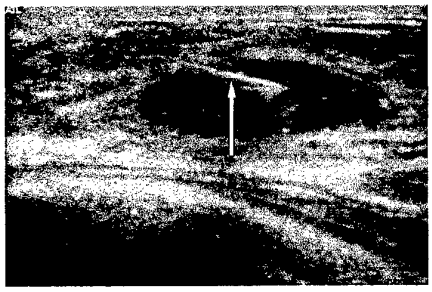
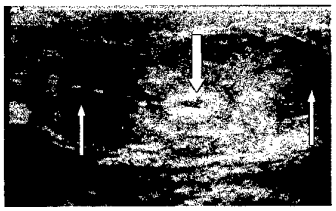


Fig 7



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The role of intra-operative cytology in determining the histological status of the sentinel lymph node.

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Department of Surgery,[1] Institute of Nuclear Medicine, [3]Department of Histopathology, Royal Free and University College Medical School, UCL, London and [2] Department of Surgery, Norfolk and Norwich NHS Trust, UK

Aims: The histological status of the axillary lymph node remains one of the most important prognostic indicators in breast cancer patients. It was the aim of this study to evaluate the accuracy of touch imprint cytology (TIC) as an intra-operative diagnostic tool to determine the histological status of the sentinel lymph node (SLN) in patients with invasive breast cancer.

Methods: Between September 1997 and February 2000, a total of 117 patients were enrolled in this study. The study was approved by the local ethics committees. The median age of the patients was 58 years (range: 34-83). After harvesting, the SLN was bisected and a clean glass slide touched onto the cut surface, resulting in cell imprints. Three slides were prepared per SLN and underwent rapid, definitive and immunohistochemical staining.

Results: A total of 141 SLN were biopsied from 117 patients (1.2 nodes per patient). The pathological diagnosis by TIC was accurate in 115 patients (98.2 per cent). In 30 patients there was evidence of metastastic deposit on definitive histology (25.6 per cent) and TIC correctly diagnosed this in 28 patients,. There were no false-positive cases in this series. With two false-negative cases, the sensitivity of this technique was 93.7 per cent. The specificity was 100 per cent and the positive predictive accuracy, 100 per cent and negative predictive accuracy, 97.7 per cent.

Conclusions: TIC is a rapid and reliable intra-operative method for determining the histological status of the SLN in patients with breast carcinoma. It will enable the surgeon to decide on performing ALND at the time of initial surgery with acceptable accuracy. Immunocytochemistry may be used to improve diagnostic accuracy of this method.

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8.5 INTRA-OPERATIVE ASSESSMENT BY OPTICAL BIOPSY FOR SENTINEL LYMPH NODE METASTASIS IN BREAST CANCER

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Aims The histological status of the axillary lymph nodes remains one of the most important prognostic indicators in breast cancer patients. It was the aim of this study to evaluate the accuracy of optical biopsy[1] (OB) as an intraoperative diagnostic tool to determine the histological status of the sentinel lymph node (SLN) in patients with invasive breast cancer.

Procedures Since October 1998, A total of 51 patients had been enrolled in the second phase of this study. The median age of the patients was 52 years (range, 34 to 88 years). After harvesting, the SLN was bivalved. Optical spectra were acquired using a clean probe from a number of representative points on the cut surface. The SLN was sent for histopathology.

Results A total of 77 SLN were biopsied from 51 patients (1.5 SLN per patient). The sensitivity of this technique was 87.1%; the specificity was 85.2%.

Significance Current intra-operative methods of assessing SLN for metastasis in breast cancer are fresh frozen section and imprint cytology. These techniques are operator dependent and time consuming. OB has the potential to provide an instant, non-operator dependent assessment of sentinel nodes.

Conclusion OB has the potential to provide instant and non-operator dependent intra-operative analysis of SLN in patients with breast cancer, which will enable the surgeon to decide on performing axillary lymph node dissection at the time of initial surgery. Sensitivity and specificity should increase as the database of correlated biopsies increase in size.

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The role of dynamic imaging in sentinel biopsy in breast cancer.

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The aim of this study is to evaluate and to define the role of dynamic imaging in sentinel node (SN) biopsy for breast cancer.

73 patients with T1/T2, N0 lesions were investigated. Each patient received a subdermal injection of 15 MBq ^{99m}Tc nanocolloid. Anterior oblique (AO) dynamic imaging commenced immediately for 45 minutes (min.). Imaging data for each patient was reformatted into various image files: 90x10 seconds frames, 15x1 min. frames, 45x1 min. frames, 3x5min. frames, 9x5 min. frames. Patterns of uptake were analysed using the sequences of dynamic frames. Time-(radio)activity curve (TAC) analysis was performed when visual interpretation was problematic.

Critical study of the dynamic dataset showed all AO image information is present within the first 15 minutes post injection. In 90% of patients, the first 5 minutes imaging was adequate. In 8 patients, additional active foci were identified as second sentinel nodes (SN) or echelon nodes. This was achieved by interpreting the dynamic images alone (6) or by additional TAC analysis (2). In 6 patients, sites of uptakes were confirmed as transient.

Dynamic image acquisition does not require to extend beyond 15 minutes. I min. framing offers optimal imaging format. The role of dynamic imaging is to distinguish true SNs from transient hotspots and second echelon nodes.

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INTRA-OPERATIVE ASSESSMENT BY OPTICAL BIOPSY FOR SENTINEL LYMPH NODE METASTASIS IN BREAST CANCER.

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[1]National Medical Laser Centre, [2]Department of Surgery, [3]Department of Histopathology, [4]Institute of Nuclear Medicine, Royal Free and University College Medical School, University College London; [4]Department of Biomedical Engineering, University of Boston, USA

Background:
Intra-operative assessment of sentinel lymph node (SLN) enables the surgeon to decide on immediate axillary lymph node dissection at the time of SLN biopsy. The aim of this study is to develop a device (Optical Biopsy System, OBS) capable of assessing lymph nodes (LN) for metastasis in patients with invasive breast cancer, that can be used intra-operatively, using the principles of Elastic Scattering Spectroscopy (ESS).

Methods:
ESS involves the spectral analysis of light scattering by intra- and extra-cellular components, and has previously been demonstrated to be sensitive to morphological changes of tissue.

The optical biopsy system consists of a white light source, a parallel pair of optical fibres, a spectrometer and a computer. The process involves the delivery of light from the light source directly onto the sectioned surface of LN via one of the optical fibres. After having been scattered by the tissue, the light is collected by the second optical fibre, and is analysed by the spectrometer and the computer, generating an optical spectrum. The entire process takes less than one second.

The optical spectrum was further analysed against a larger and independent training set of optical spectra from LN using model based analysis to determine the status of LN. The result was compared the histological findings.

Results:
In total, 75 LN were tested. OBS provided diagnosis in 72 LN (3 LN were deemed indeterminate), and was correct in 62 LN. OBS was able to correctly identify metastasis in 16 out of 19 LN compared with histology. There were 7 false positives and 3 false negatives, thus giving a sensitivity of 84.2% and specificity of 86.8%.

Conclusion:
Current intra-operative techniques to assess SLN are imprint cytology and fresh frozen section, which are time consuming and operator dependent. OB has the potential to provide instant and non-operator dependent intra-operative analysis of SLN in patients with breast cancer; sensitivity and specificity should increase as the database of correlated biopsies increase in size.

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ORAL

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Elastic scattering spectroscopy for intraoperative detection of sentinel lymph node metastasis

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Background: Intra-operative assessment of sentinel lymph node (SLN) enables the surgeon to decide on immediate axillary lymph node dissection at the time of SLN biopsy. The aim is to develop a device using the principles of Elastic Scattering Spectroscopy (ESS) capable of intraoperative detection of SLN metastasis in patients with invasive breast cancer.

Methods: ESS involves the spectral analysis of light scattering by intra- and extra-cellular components, and has previously been demonstrated to be sensitive to morphological changes of tissue.

The ESS system consists of a white light source, a parallel pair of optical fibres, a spectrometer and a computer. The process involves the delivery of light from the light source directly onto the sectioned surface of LN via one of the optical fibres. After having been scattered by the tissue, the light is collected by the second optical fibre, and is analysed by the spectrometer and the computer, generating an optical spectrum. The entire process takes less than one second.

The optical spectrum was further analysed against a larger and independent training set of optical spectra from LN using model based analysis to determine the status of LN. The result was compared the histological findings.

Results: In total, 75 LN were tested. ESS provided diagnosis in 72 LN (3 LN were deemed indeterminate), and was correct in 62 LN. ESS was able to correctly identify metastasis in 16 out of 19 LN compared with histology. There were 7 false positives and 3 false negatives, thus giving a sensitivity of 84.2% and specificity of 86.8%.

Conclusion: Current intra-operative techniques to assess SLN are imprint cytology and fresh frozen section, which are time consuming and operator dependent. ESS has the potential to provide instant and non-operator dependent intra-operative analysis of SLN in patients with breast cancer; sensitivity and specificity should increase as the database of correlated biopsies increase in size.

POSTER

83

The role of dynamic imaging in sentinel lymph node biopsy in breast cancer

A. Lee[1], M. Keshtgar[2], W. Waddington[1], P. Ell[1]. [1] *Royal Free and University College Medical School, University College London, Institute of Nuclear Medicine;* [2] *Royal Free and University College Medical School, University College London, Department of Surgery*

Introduction and Aim: The use of radiopharmaceutical in sentinel lymph node (SLN) biopsy enables preoperative visualisation of SLN. However, the precise role of preoperative imaging remains undefined in SLN biopsy for breast cancer. Furthermore, there has been no published study to date in evaluating the role of dynamic imaging. The aim of this study is to evaluate the role of high resolution, early dynamic imaging in SLN biopsy for breast cancer, primarily through direct visualisation and analysis of radiopharmaceutical transit through lymphatic tracts.

Methods: Patients with T1/T2, N0 invasive breast cancer underwent SLN localisation using intra-dermal injection of 15 MBq of 99mTc-nanocolloid. Gamma camera anterior-oblique dynamic imaging commenced simultaneously with tracer administration for 45 minutes, and was followed by anterior and lateral static imaging. Dynamic imaging data was reformatted into image files of different time frames. Patterns of uptake were analysed using the sequences of dynamic frames and time activity curve.

Results: SLN localisation was successful in 70/73 studies (95.9%) in 72 patients. Imaging information was present within the first 15 minutes of dynamic imaging in 67/70 studies (95.7%). Critical analysis of dynamic data helped to differentiate true SLN from secondary echelon nodes in 8 studies and transient foci of radioactivity in 6 studies. In 17 studies, SLN contained metastatic disease. The detection of SLN metastasis was independent from the use of dynamic imaging.

Conclusion: Dynamic imaging improves the interpretation of preoperative SLN imaging for breast cancer, but does not contribute significantly to the successful detection of SLN. Hence preoperative dynamic imaging is not necessary in SLN biopsy for breast cancer.

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Breast 19

Elastic scattering spectroscopy for the diagnosis of breast cancer

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Introduction: Elastic scattering spectroscopy (ESS) studies tissue structure by spectral analysis of light absorption and scattering by intra- and extracellular components of tissue, and has previously been shown to be sensitive to morphological changes in tissue. The aim of our study is to determine the accuracy of ESS in diagnosis of breast cancer.

Methods: ESS system consists of a white light source, a parallel pair of optical fibres, a spectrometer and a computer. The process involves the delivery of light from the light source directly onto breast tissue via an optical fibre. The light is collected by the second optical fibre, and is analysed by the spectrometer and the computer, generating an optical spectrum. The entire process takes less than 1 s. Using model base analysis, the optical spectrum was analysed against an independent set of optical spectra taken from normal and malignant breast tissue to provide the diagnosis. The result was compared to the histological findings of the breast tissue.

Result: Optical spectra were taken from 94 breast tissues. ESS provided correct diagnosis in 87 out of 88 breast tissues (six were deemed indeterminate). ESS was able to diagnose 15 out of 16 breast cancers. There was one false positive, thus giving a sensitivity of 93.8 per cent, specificity of 98.6 per cent, positive predictive value 93.8 per cent and negative predictive value 100 per cent.

Conclusion: ESS has the potential to provide an instant and nonoperator-dependent detection of breast cancer, which can be used to guide core biopsies and to assess breast lesions and tumour margins intraoperatively.

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Abstract No 35

Optical Biopsy: The Technique and Experience in determining Lymph Node Status in Breast Cancer

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Introduction

Optical Biopsy (OB) using Elastic Scattering Spectroscopy (ESS) utilises the differential light absorption and scattering properties of tissue for diagnostic purposes. This technique involves spectral analysis of light scattered by intra- and extra-cellular components. Previous studies have demonstrated sensitivity to the morphological changes that occur in these components between malignant and non-malignant tissues. The aim of this study is to determine the accuracy of OB-ESS in detecting lymph node (LN) metastasis in breast cancer.

Methods

The OB-ESS system consists of a pulsed xenon arc-lamp, a parallel pair of optical fibers, a spectrometer and a lap-top computer. Light between 320-900nm is delivered directly onto the tissue surface via an optical fiber, and is subsequently collected by the second optical fiber. The scattered light is detected using a spectrometer, generating an optical spectrum, which is subsequently analyzed by statistical software to provide diagnosis. The entire process takes one second per optical spectrum and is non-operator dependent.

Optical spectra were acquired from the sectioned surface of 327 axillary LN or sentinel lymph nodes (SLN) obtained from 136 breast cancer patients. Subsequent to OB, LN underwent routine histological examination with additional immunohistochemistry for SLN. Linear discriminant analysis (LDA) with separate testing and training sets was used to differentiate between optical spectra of metastatic and normal LN.

Results

It was shown that OB-ESS can be used intra-operatively by surgeons under sterile conditions. A total of 2804 optical spectra were obtained from sites on individual LN with a median of 6 sites per LN. 1829 of these were randomly selected as the training set. Once the LDA has established a discriminant algorithm, this was tested on the remaining data giving an accuracy of 81% for differentiating positive and negative LN (Sensitivity 69%, Specificity 85%). When a subset of 23 LN with close to total metastatic replacement was considered against 177 normal LN, the accuracy was found to be 96%(sensitivity of 70% and specificity of 99%).

Conclusion

OB-ESS has been demonstrated to have sensitivity and specificity broadly in line with those of current intra-operative techniques namely imprint cytology and fresh frozen section in examining SLN for metastasis. The advantage of OB-ESS is that it is less time consuming and is non-operator dependent. Further clinical evaluation should validate the application in SLN biopsy.

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Comparative Study of Imprint Cytology and Elastic Scattering Spectroscopy in assessing Sentinel Lymph Nodes status in Breast Cancer

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Introduction

Intra-operative assessment of sentinel lymph nodes (SLN) would enable an immediate clinical decision on axillary dissection. Touch imprint cytology (TIC) has been widely evaluated for this purpose. Elastic Scattering Spectroscopy (ESS) utilizes the differential light absorption and scattering properties of tissue. Recent studies have demonstrated its sensitivity in determining lymph node status in breast cancer. The aim of this study is to compare the accuracy of TIC and ESS in determining SLN status.

Methods

The ESS system consists of a light source, an optical fiber probe and a spectrometer enabling analysis of light scattered back from the sectioned surface of SLN. Linear discriminant analysis (LDA) with separate testing and training sets was used to differentiate between optical spectra of positive and negative SLN.

115 SLN were harvested from 68 patients. Each SLN was bisected along its longitudinal axis. Optical measurements were acquired using the ESS system. In 89 SLN, TIC was also obtained by gentle touching of the sectioned SLN surface onto 3 glass slides for rapid, definitive and immunocytochemistry staining. All SLN underwent histological assessment with immunohistochemistry.

Results

A total of 1210 optical spectra were obtained from a median of 7 sites per SLN. 805 of these were randomly selected as the training set. Once the LDA has established a discriminant algorithm, this was tested on the remaining 405 optical spectra giving an accuracy of 87% for differentiating positive and negative lymph nodes (Sensitivity 73%, Specificity 92%).

In the 89 SLN which also had TIC, 8 SLN contained metastasis. When all individual optical spectra of the same SLN were used to provide an overall SLN status and equal number of positive and negative SLN were considered, the accuracy of ESS was found to be 84% (sensitivity 75%, Specificity 93%). TIC had an accuracy of 87% (sensitivity of 75% and specificity of 99%). A students t-test on overall accuracy and Kappa value for TIC and ESS indicated equivalence at the 95% confidence level.

Conclusion

ESS has equivalent sensitivity and specificity to TIC for determining SLN metastasis. The advantage of ESS is that it provides an almost instant analysis of SLN without the need of expert interpretation.

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Abstract Accepted for Presentation –Date to be confirmed

Optical Biopsy: The Technique and Experience in determining Lymph Node Status in Breast Cancer

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Introduction

Optical Biopsy (OB) using Elastic Scattering Spectroscopy (ESS) utilizes the differential light absorption and scattering properties of tissue for diagnostic purposes. This technique involves spectral analysis of light scattered by intra- and extra-cellular components, and is sensitive to the morphological changes in malignant tissues. The aim of this study is to determine the accuracy of OB-ESS in detecting axillary lymph node (LN) metastasis in breast cancer.

Methods

The OB-ESS system consists of a xenon arc-lamp, a parallel pair of optical fibers, a spectrometer and a computer. Light is delivered directly onto the tissue surface via an optical fiber, and is subsequently collected by the second optical fiber. The scattered light is detected using a spectrometer, generating an optical spectrum, which is subsequently analyzed by statistical software to provide diagnosis. The entire process takes one second per optical spectrum.

Optical spectra were acquired from the sectioned surface of 327 axillary LN or sentinel lymph nodes (SLN) obtained from 136 breast cancer patients. Subsequent to OB, LN underwent routine histological examination with additional immunohistochemistry for SLN. Linear discriminant analysis (LDA) with separate testing and training sets was used to differentiate between optical spectra of positive and negative LN.

Results

OB-ESS can be used intra-operatively by surgeons under sterile conditions. A total of 2804 optical spectra were obtained from sites on individual LN (median of 6 sites per LN). 1829 of these were randomly selected as the training set. Once the LDA has established a discriminant algorithm, this was tested on the remaining data giving an accuracy of 81% for differentiating positive and negative LN (Sensitivity 69%, Specificity 85%). When a subset of 23 LN with close to total metastatic replacement was considered against 177 normal LN, the accuracy was found to be 96% (Sensitivity 70%, Specificity 99%).

Conclusion

In examining lymph nodes, OB-ESS appears to have sensitivity and specificity broadly in line with those of current intra-operative techniques namely imprint cytology and fresh frozen section. The advantage of OB-ESS is that it is less time consuming and is non-operator dependent. Further clinical evaluation should validate the application in SLN biopsy.

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CLINICAL AND PHYSICAL PERFORMANCE ASSESSMENT OF A SELF-CONTAINED, HAND-HELD GAMMA PROBE

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Introduction: We have evaluated a compact gamma probe for SLN detection (HandheldGammaFinder [HGF], Silicon Instruments GmbH). The unit is self-contained with an internal battery, forward collimation and shielding, fixed 120 keV energy threshold, single-range audio signal, LCD panel for countrate display and controls to modify detector setup. Battery life is 300 hrs, with manufacturer exchange.

Materials and Methods: Physical and clinical performance was assessed. Clinically, ex-vivo measurements of SLN activity and in-vivo injection site counts were recorded against those for an uncollimated Neoprobe 1500 (Ethicon [N1500]) for 18 ca. breast and 2 melanoma patients administered 12-20 MBq ^{99m}Tc Nanocoll (Amersham Health) 24 hrs before excision.

Results: Mean HGF ex-vivo SLN counts were 8.7±4.4 % that for N1500, mean injection site counts were 4.7±5.6 %. Sensitivity for an on-axis 40 ul ^{99m}Tc point source in contact with the probe face was 4.79 cps/kBq [HGF] and 20.3 cps/kBq [N1500]. ^{99m}Tc shield penetration was <0.1 % [HGF] and 0.5 % [N1500]. Spatial resolution for a 1 mm line source at 10 mm depth in perspex (tissue equivalent medium) was 11 mm [HGF] and 95 mm [N1500] full width tenth maximum. A detectability task to localise obscured simulated SLN sources containing 37.5 [A], 12.5 [B], 3.75 [C], 1.25 [D] kBq (equal to 3, 1, 0.3, 0.1 % 20 MBq injected activity ^{99m}Tc @ 24 hrs p.i.) demonstrated :

	detected with HGF	detected with N1500
SLN at surface	A B	A B C D
SLN at 10mm depth	A (B)	A B C (D)
SLN at 30mm depth	A	A B

Conclusions: HGF has lower sensitivity than N1500 due to a smaller detector aperture and 120 keV energy threshold, and a narrower field of response. The effect of these factors together reduce its ability to detect weaker and/or deeper SLNs with respect to the more sensitive and less directional N1500.

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A NOVEL 'OPTICAL BIOPSY' TECHNIQUE USING ELASTIC SCATTERING SPECTOSCOPY FOR DYSPLASIA AND CANCER IN BARRETT'S OESOPHAGUS

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Background: Barrett's oesophagus develops through dysplasia into adenocarcinoma. Surveillance for dysplasia using conventional white light endoscopy requires histological examination of multiple random biopsies, and has a poor diagnostic yield. Recently, fluorescence endoscopy has become available to detect abnormal areas for targeting biopsy. Elastic scattering spectroscopy (ESS) is another novel technique for minimally invasive optical diagnosis of tissue pathology. It is sensitive to size and structure of sub-cellular components and is complementary to fluorescence endoscopy as a form of real-time *in vivo* optical biopsy. We present our preliminary data using this technology to diagnose dysplasia or cancer within Barrett's oesophagus. **Patients & Methods:** 108 elastic scattering spectra ('optical biopsy', 2-3 per site) and matched oesophageal biopsies were taken from 41 sites in 27 patients with Barrett's metaplasia. A pulse of white light from a xenon arc lamp was passed down a probe the size of normal biopsy forceps that contained an optical fibre in direct contact with the tissue being interrogated and an adjacent fibre to detect scattered light. Histologically, biopsies were defined as normal, dysplastic or carcinoma. Optical biopsies took less than 1 second to perform. Analysis of spectra between 350 and 750 nm was carried out using artificial neural networks (ANN). **Results:** Histological findings were correlated with appropriate spectra. A set of 86 spectra from non-dysplastic, dysplastic or cancerous areas was used as a learning set and was analysed in 15 discrete wavelength bands. 12 other spectra from non-dysplastic and 10 from dysplastic or cancerous areas were tested using the ANN: specificity 100%, sensitivity 80%, positive predctive value: 90%. **Conclusions:** Preliminary results using ESS show that this technique has potential as a real time diagnostic test for *in vivo* diagnosis of dysplasia or cancer within Barrett's mucosa.

British Society of Dermatology, July 2002 Plenary Presentation

OPTICAL BIOPSIES IN THE DIAGNOSIS OF PIGMENTED LESIONS: COMPARISON WITH CLINICAL AND HISTOPATHOLOGICAL DIAGNOSIS

Scarisbrick JJ, CDO Pickard, AC Lee, GM Briggs, SG Bown, MRS Keshtgar, R Yu.

Successful management of malignant melanoma(MM) depends on early detection and diagnostic accuracy. However studies have found a diagnostic accuracy of MM amongst dermatologists of only 80% compared with histological diagnosis with poorer results from family practitioners. Optical biopsy is a non-invasive procedure which utilises elastic scattering spectroscopy(ESS). A related technique, reflectance spectrometry, may discriminate between benign and malignant pigmented lesions in vivo[1]. We have therefore assessed ESS in the diagnosis of pigmented lesions and compared the results to both clinical and histopathological diagnosis.

Ninety-three pigmented lesions from 74 patients attending our pigmented lesion clinic since 2000 or seen at our Melanoma Day, June 2000, were examined clinically and divided into benign, dysplastic or malignant lesions. Optical spectra were acquired at several points from each lesion and adjacent normal skin. Lesions were then excised and sent for histological examination.

Lesions were characterised histologically into 11 MM(3 in-situ), 13 dysplastic naevi and 52 benign naevi(17 clinically benign naevi were not excised). Clinical examination had a sensitivity of 73% and specificity of 93% at detecting MM from other pigmented lesions the sensitivity increased to 92% when differentiating both MM and dysplastic naevi from benign naevi but was less specific(58%). Optical biopsies classified by linear discriminant analysis indicated a sensitivity for detection of MM from benign naevi of 91% with a specificity of 79%. Analysis could also distinguish MM from dysplastic naevi with a sensitivity of 92% and specificity of 79%.

These data suggest that optical biopsies are capable of differentiating MM, dysplastic and benign naevi with a high sensitivity and provide adjuvent information to clinical examination by a dermatologist which could be useful in the triage of pigmented lesions.

[1]Marchesini R et al: Photochem Photobiol.53:77-84,1991.

US PATENT & TRADEMARK OFFICE

PATENT APPLICATION FULL TEXT AND IMAGE DATABASE

United States Patent Application **20020137993**
Kind Code **A1**
Pickard, David **September 26, 2002**

Spectrum processing and processor

Abstract

An elastic scattering spectrum taken from human tissue is taken and preprocessed by dividing the absorption spectrum into a plurality of wavelength ranges and fitting the measured absorption spectrum to the absorption of predetermined absorption components in some fitting ranges and to a smooth function, such as a straight line, in the remainder of the fitting ranges. The absorption components may include, for example, haemoglobin.

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Goverment Interests

[0001] This invention was made with Government support under DAMD17-98-1-8343 awarded by the US Army Medical Research Acquisition Activity. The Government has certain rights in the invention.

Foreign Application Data

Date	Code	Application Number
Feb 7, 2001	GB	0103030.3

Claims

1. A method of processing a broadband elastic scattering spectrum obtained from tissue comprising the steps of: obtaining, in a plurality of fitting ranges of wavelength, fitting parameters giving the best fit to the spectrum in the respective fitting ranges; and recording the fitting parameters as a parameter data set representing the spectrum; wherein in at least one fitting range, but not all fitting ranges, the fit is to the absorption of at least one predetermined component; and in the remainder of the fitting ranges, the fit is to a smooth function.

2. A method according to claim 1 wherein the fit to a smooth function is to a straight line.

3. A method according to claim 1 wherein the plurality of fitting ranges, taken together, include at least 60% of the wavelength range of the complete broadband elastic scattering spectrum.

4. A method according to claim 1 wherein in at least one fitting range, the fit to the absorption of at least one predetermined component is to the absorption line shape of the at least one predetermined absorbing component.

5. A method according to claim 4 wherein the fit to the absorption line shape of the at least one predetermined absorbing component uses a parabolic approximation.

6. A method according to claim 1 wherein the fit to the absorption of at least one predetermined component is a fit to an absorption spectrum previously measured using an optical biopsy probe on a sample of the predetermined absorbing component in a tissue-like matrix.

7. A method according to claim 1 further comprising, after the step of obtaining fitting parameters in one fitting range, calculating a modified spectrum to compensate for the shape of the spectrum represented by the fitting parameters in that fitting range, and using the modified spectrum when fitting parameters in at least one further fitting range.

8. A method according to claim 7 wherein after obtaining fitting parameters representing a fit to an absorption line shape in one fitting range, a modified spectrum is calculated by inputting the recorded fitting parameters in that fitting frequency range into a model of the absorption, calculating the expected absorption spectrum determined by the model with the input fitting parameters and subtracting the calculated absorption spectrum from the initial spectrum to obtain the modified spectrum used when fitting parameters in at least one further fitting range.

9. A method according to claim 1 wherein, in at least one fitting range, the predetermined absorbing components are haemoglobin in oxygenated and deoxygenated forms.

10. A method according to claim 9 wherein the fitting range in which the predetermined absorption components are oxygenated and deoxygenated haemoglobin is at least part of the range from 320 nm to 620 nm.

11. A method according to claim 1 wherein in one fitting range in the range 400 to 520 nm the predetermined absorbing component is beta-carotene.

12. A method according to claim 1 including calculating absorption of other chromophoric components in the tissue which share haemoglobins Soret absorption band around 412 and 430 nm and recording the differential absorptions as fitting parameters.

13. A method according to claim 12 wherein the chromophoric components include cytokines.

14. A method according to claim 1 wherein in a fitting range including at least part of the range from 230 nm to 512 nm the predetermined absorbing component is a bile pigment.

15. A method according to claim 1 wherein in one fitting range covering substantially the whole of the spectrum the predetermined absorbing component is melanin.

16. A method according to claim 1 including fitting in a range below 350 nm to protein absorption.

17. A method according to claim 1 wherein in one fitting range in the predetermined absorbing component is an exogenous dye.

18. A method including recording an elastic scattering spectrum from tissue, and preprocessing the spectrum using a method in accordance with claim 1.

19. A method comprising the steps of: recording an elastic scattering spectrum from tissue; processing the spectrum to produce a number of parameters characterising the spectrum; determining to which, if any, of a number of classes the parameterised spectrum belongs by measuring the distance in a multidimensional parameter space between the parameters characterising the

spectrum and parameters characterising the classes; and outputting the class, if any, to which the spectrum is determined to belong.

20. A method according to claim 19 wherein the step of determining to which, if any class the parameterised spectrum belongs uses classes determined by hierarchical cluster analysis.

21. A computer program product for loading into a computer arranged to cause the computer to carry out the steps of: obtaining, in a plurality of fitting ranges of wavelength, fitting parameters giving the best fit to the spectrum in the respective fitting ranges; and recording the fitting parameters as a parameter data set representing the spectrum; wherein in at least one fitting range, but not all fitting ranges, the fit is to the absorption of at least one predetermined component; and in the remainder of the fitting ranges, the fit is to a smooth function.

22. Apparatus for analysing an elastic scattering spectrum taken from tissue, comprising: a data store for recording the elastic scattering spectrum; a processor arranged to obtain a plurality of fitted parameters characterising the spectrum by carrying out the steps of: obtaining, in a plurality of fitting ranges of wavelength, fitting parameters giving the best fit to the spectrum in the respective fitting ranges; and recording the fitting parameters as a parameter data set representing the spectrum; wherein in at least one fitting range, but not all fitting ranges, the fit is to the absorption of at least one predetermined component; and in the remainder of the fitting ranges, the fit is to a smooth function.

23. Apparatus for elastic scattering spectroscopy of tissue, including a light source for emitting light in a broad frequency band; a probe for transmitting light from the light source to tissue and for receiving light scattered in the tissue; a spectrometer for measuring the spectrum of the received light as a function of frequency; and a processor for processing the measured light spectrum arranged to obtain a plurality of fitted parameters characterising the spectrum by carrying out the steps of: obtaining, in a plurality of fitting ranges of wavelength, fitting parameters giving the best fit to the spectrum in the respective fitting ranges; and recording the fitting parameters as a parameter data set representing the spectrum; wherein in at least one fitting range, but not all fitting ranges, the fit is to the absorption of at least one predetermined component; and in the remainder of the fitting ranges, the fit is to a smooth function.

24. Apparatus according to claim 23 wherein the probe contains a first optical fiber bringing light from the light source to a probe tip; and a second optical fiber bringing scattered light from the probe tip to the spectrometer; wherein the ends of the first and second fibers at the probe tip are arranged adjacently spaced apart by a predetermined distance.

25. Apparatus according to claim 23 further comprising a decision processor for checking the fitted parameters against the results for one or more predetermined medical conditions and outputting the best fit medical condition based on the decision processor output.

26. Apparatus according to claim 25 wherein the decision processor uses hierarchical cluster analysis.

27. A training method comprising the steps of: recording a plurality of broadband elastic scattering spectra from patients for which it is known whether they have a predetermined medical condition; obtaining, in a plurality of fitting ranges of wavelength, fitting parameters giving the best fit to the spectrum in the respective fitting ranges; and recording the fitting parameters as a parameter data set representing the spectrum; wherein in at least one fitting range, but not all fitting ranges, the fit is to the absorption of at least one predetermined component; in the remainder of the fitting ranges, the fit is to a smooth function; and training a discriminant model using the preprocessed spectra.

Description

FIELD OF THE INVENTION

[0002] The invention relates to a method of processing a spectrum, in particular an elastic scattering spectrum taken from tissue and to apparatus including a spectrum processor for carrying out the method.

BACKGROUND ART

[0003] Elastic scattering spectroscopy is a known technique for investigating tissue. In essence, light is shone into human tissue, generally living human tissue, and a photoreceptor measures the light transmitted to the photoreceptor through scattering in the tissue. The spectrum of light passing through the tissue is then recorded, and used to assist in diagnosis of any of a number of medical conditions that the patient may have. Thus, the technique may be described as optical biopsy.

[0004] Prior art apparatus for carrying out optical biopsy is presented in WO98/27865 to David Benaron, and in U.S. Pat. No. 5,303,026 to Stroble et al. The latter patent describes a system having a light source feeding into a reference optical fiber and a probe optical fiber. The probe optical fiber being brought to a probe tip. The probe tip has another optical fiber arranged adjacently of it, which collects light and brings it to a detection system which compares its intensity to the intensity of light on the reference optical fiber. When the probe tip is brought against human tissue the detection system can record the difference

as a between the reference signal strength and that of the light scattered by the human tissue as a function of wavelength to obtain an optical biopsy spectrum.

[0005] The use of an elastic scattering spectrum to diagnose a number of medical conditions is described in a number of papers. Zhengfang GE et al describe in the paper "Identification of Colonic Dysplasia and Neoplasia by Diffuse Reflectance Spectroscopy and Pattern Recognition techniques". Applied Spectroscopy Volume 52 number 6 (1998) p 833, a method of identifying colonic dysplasia and neoplasia. The paper describes a number of different pattern recognition techniques used to evaluate the samples.

[0006] One of these is multiple linear regression analysis, which is used to fit to reflectance intensities measured at 26 different wavelengths every 16 nm in the range 350 nm to 750 nm. An output score is obtained from the formula: 1 score = k + j = 1 26 a j D i (j)

[0007] The coefficients a are fitted coefficients arranged such that the score is +1 for adenomatous polyps and -1 for hyperplastic polyps. D.sub.I.lambda..sub.I is the reflectance value for the ith tissue sample at the jth wavelength.

[0008] Another approach described in the paper by Zhengfang et al is linear discriminant analysis. This is a method of classifying a test into one of k groups using a classification score that can be computed from a formula. The test is classified into the group which gives the lowest classification score.

[0009] The classification of a test object X.sub.I=(x.sub.1, x.sub.2, . . . x.sub.d) containing d independent integers is assigned to one of k gropus using the classification score defined as

D.sub.k.sup.2=(X.sub.I-.mu..sub.k).sup.TM.sup.-1(X.sub.I-.mu..sub.k)

[0010] where M.sup.-1 is the inverse of the pooled covariance matrix over all classes 2 M = 1 n k i = 1 n k (X i - k) (X i - k) T .

[0011] A third approach is backpropagating neural network analysis using a multilayer neural network with n input nodes, a hidden layer and an output layer. Neural network techniques have been widely reported and will not be discussed further here.

[0012] Other papers describe the use of elastic scattering spectroscopy in the diagnosis of a number of conditions. Backman et al describe the detection of precancerous epithelial cells in "Detection of Preinvasive Cancer Cells", Nature, vol 406 p35 (2000). Perelman et al, in "Observation of Periodic Fine Structure in Reflectance from Biological Tissue: A New Technique for Measuring Nuclear Size Distribution", Phys. Rev. Lett. vol 80 p627 (1998) describe periodic fine structure in mucosal membranes. The diagnosis of bladder cancer is described in "Spectroscopic Diagnosis of Bladder Cancer with Elastic Light Scattering" Mourant et al, Lasers in Surgery and Medicine, Volume 17 page 350 (1995). The use of elastic scattering to diagnose pathologies in the gastrointestinal tract is described in "Elastic Scattering Spectroscopy as a diagnostic tool for differentiating pathologies in the Gastrointestinal tract: preliminary testing", Mourant et al, Journal of Biomedical optics, Vol 1 p192, and in "Ultraviolet and visible spectroscopies for tissue diagnostics: fluorescence spectroscopy and elastic scattering spectroscopy", Bigio and Mourant Phys. Med. Biol. Volume 42 p803 (1997).

[0013] It is thus clear that the use of elastic scattering spectroscopy is attracting interest as a diagnostic tool. In spite of this research interest the most reliable approach presently used for detection of cancer in tissue and other conditions is histology. However, this is time consuming and laborious and in many situations, especially for diagnosing cancer, multiple biopsies may be needed.

[0014] There is thus a need to develop optical techniques further. One application is to guide conventional biopsies, avoiding false negatives and reducing the number taken while increasing yield. The long-term goal is to develop the techniques to a point where they can be used rapidly, efficiently and reliably to diagnose conditions without the need for histology.

SUMMARY OF THE INVENTION

[0015] According to the invention there is provided a method of processing a broadband elastic scattering spectrum obtained from tissue comprising the steps of: obtaining, in a plurality of fitting ranges of wavelength, fitting parameters giving the best fit to the spectrum in the respective fitting ranges; and recording the fitting parameters as a parameter data set representing the spectrum; wherein in at least one fitting range the fit is to the absorption of at least one predetermined component, and in the remainder of the fitting ranges the fit is to a smooth function.

[0016] By fitting in a number of different fitting regions to known absorption spectra and to a smooth function a measured spectrum including a large number of data points can be reduced to the very much smaller number of data points, i.e. the fitting parameters. Subsequent data processing using the fitting parameters instead of the whole spectrum (as used in the prior art discussed above) may allow simpler, more reliable and more rapid assessment of the patient's condition.

[0017] The method can be thought of as using model dependent fitting, i.e. of analysing the spectrum using a model of the absorption with certain absorbing components absorbing at certain frequencies before carrying out any diagnosis or

discrimination.

[0018] The fit to the absorption of at least one predetermined component may be to the absorption line shape of the at least one predetermined absorbing component. In particular, the fit may use a parabolic approximation to the peak of absorption of that absorbing component.

[0019] The fit to the absorption of at least one predetermined component may be a fit to an absorption spectrum previously measured using an optical biopsy probe on a sample of the predetermined absorbing component in a tissue-like matrix. This absorption spectrum, in general, differs from the simple absorption spectrum due to scattering obtained from a conventional optical transmission cell and available in most textbooks.

[0020] The use of a spectrum measured using an optical biopsy probe on a sample in a tissue-like substrate has not previously been suggested, as far as the inventor is aware.

[0021] Alternatively, especially for single component systems, the fit may be nothing more than determining the excess of absorption in the spectrum at a predetermined frequency over the background spectral lineshape due to scattering calculated by extrapolating a straight line fit in a neighbouring region of the spectrum. The predetermined frequency is preferably the peak absorption wavelength of the absorbing component.

[0022] The fit to a smooth function is preferably to a straight line. Such fits are straightforward to carry out and with suitable choice of fitting ranges can parameterise an absorption slowly varying with wavelength, i.e. the spectral lineshape due to scattering in the absence of any absorption features.

[0023] The fitting ranges, taken together, preferably include at least 60%, further preferably 80% of the wavelength range of the complete broadband elastic spectrum, at least in the range in which the spectrum has been measured with reasonable accuracy. In this way substantially all of the measured spectrum may be parameterised.

[0024] The method may also include, after obtaining fitting parameters in one fitting range, calculating a modified spectrum to compensate for the shape of the spectrum represented by the fitting parameters in that fitting range, and using the modified spectrum when fitting parameters in at least one further fitting range. This may be done by inputting the recorded fitting parameters in that fitting frequency range into a model of the absorption, calculating the expected absorption spectrum determined by the model with the input fitting parameters and subtracting the calculated absorption spectrum from the initial spectrum to obtain the modified spectrum used when fitting parameters in at least one further fitting range.

[0025] It should be noted that the fitting regions may overlap. For example, it may be desired to fit to a line shape of a known absorption component in a certain fitting region and then to multiply by a predetermined function to remove that line shape. However, there may still be information in the residual intensity of the lineshape due to scattering and this can be fitted using linear fitting parameters in a fitting region that may overlap or even be identical to the fitting region used to fit to the line shape of the absorption component.

[0026] The preprocessed spectrum may be fed to a discriminating algorithm to determine whether or not the data corresponds to one or more medical conditions. The discriminating algorithm may be trained to detect a particular medical condition or to discriminate between a number of clinically similar conditions. The training will use a number of training samples. The skilled person will realise that there are a number of suitable models with a number of variable fitting parameters for implementing the discriminating algorithm. For example, a neural network approach may be used, a linear discriminant analysis or a hierarchical cluster analysis. All of these are known per se, and the first two are, for example, discussed in the paper by Zhenzhou et al mentioned above. It is generally the case, however, that the smaller number of model dependent fitting parameters obtained using the preprocessing method according to the invention can provide a benefit whatever fitting and diagnosis approach is used.

[0027] One reason for the improvement is the reduction in the number of points in the data set for fitting. Whether using a neural network or other discriminant analysis, the large number of points in the original data set of the whole spectrum means that a large number of training samples would be needed to train any model of the results output from the preprocessor. By reducing the number of points in the data set to be fitted less training is required and the fit can be carried out more reliably.

[0028] A preferred approach is hierarchical cluster analysis in which the n parameters of the spectrum define a unique point in n-dimensional space. Clusters of points are determined-the diagnosis corresponds to identifying in which cluster a measured spectrum point lies. Hierarchical cluster analysis has the advantage that it allows a "don't know" response, if for example the measured point is located far from any of the clusters identified. This is of advantage in preventing false diagnosis in cases where no such diagnosis can be reliably made.

[0029] A number of different absorbing components can be fitted and each absorbing component will absorb in a different wavelength range and hence a different fitting range.

[0030] One example of general application in human tissue is to fit to the haemoglobin absorption; this can be done by fitting to the saturation and the total hematocrit concentration. The saturation is defined as the percentage of oxygenated haemoglobin

to the total hematocrit (oxygenated and deoxygenated haemoglobin). Such a fit to haemoglobin concentration may be carried out in a fitting range including at least part of the region of the spectrum from 320 nm to 620 nm.

[0031] Haemoglobin gives rise to an absorption feature which may dominate the spectrum in the region of 415 nm, called the Soret band. Other constituents of tissue also absorb near this wavelength and once the "normal" Hb absorption has been removed the absorption due to these features will be observed and may be fitted. The other features may be the absorption due to components such as cytokines.

[0032] Other absorbing components are relevant to a number of different kinds of spectrum. For example, to detect breast cancer it is preferred to fit to the beta-carotene absorption spectrum in the fitting range 400-520 nm.

[0033] In some studies, exogenous dye may be introduced into tissue for diagnostic purposes and accordingly the preprocessing method can include fitting to the spectrum of the dye used. For example for, for suspected cases of breast cancer, blue dye can be introduced into human tissue to trace the spread of the disease. For the dye used in studies to date, Patent V blue dye (Trade Mark), a fitting range including at least past of the range 530 nm to 720 nm is suitable.

[0034] One predetermined fitting region may be a region in the range 630 nm to 810 nm, and the fit may be a linear fit in this region. The method may also include fitting to a linear model in a number of other regions. These can include linear fitting in the range 340 nm to 360 nm, and/or the range 320 nm to 330 nm. These fittable regions will be observed after the removal of absorption features.

[0035] The spectral trace may be checked for and the spectrum rejected if the check reveals measurement errors or unsuitable data. For example, it may be advisable to check for a minimal transmitted intensity in the Soret band and to reject the spectrum if the measured transmitted intensity is substantially zero in this band. In other words, if the measured transmitted intensity is less than 10% full scale, preferably less than 5% full scale, the spectrum may be rejected. Another possibility is to check for interference from background illumination and to reject the spectrum if background illumination is too high. Further, the spectrum can be checked for contact between probe and tissue.

[0036] The invention also relates to a method including recording an elastic scattering spectrum from tissue, and preprocessing the spectrum as described above.

[0037] The tissue may be in vivo, i.e. tissue incorporated in the living human body.

[0038] The tissue may be in vitro, i.e. tissue removed from the body.

[0039] In another aspect there is provided a method comprising the steps of recording an elastic scattering spectrum; preprocessing the spectrum using a preprocessing method to obtain a plurality of fitting parameters characterising the spectrum; testing the preprocessed spectrum using a discriminant model; and outputting a result based on the model.

[0040] The result may be an output indicating to which class, if any, the recorded elastic scattering spectrum belongs. The output may thus be a diagnosis.

[0041] In embodiments, the discriminant model may be a neural network, linear discrimination, hierarchical cluster analysis or other methods as are known to those skilled in the art.

[0042] The preferred discriminant model uses hierarchical cluster analysis. This groups data into unbounded class regions permitting "not sure" diagnostic indications, rather than forcing a decision and risking a false diagnosis.

[0043] In another aspect, the invention relates to a method comprising the steps of recording an elastic scattering spectrum from tissue; processing the spectrum to produce a number of parameters characterising the spectrum; determining to which, if any, of a number of classes the parameterised spectrum belongs; and outputting the class, if any, to which the spectrum is determined to belong.

[0044] In a further aspect there is provided a training method comprising the steps of recording a plurality of optical biopsy spectra from tissue for which it is known whether the tissue displays a predetermined medical condition; preprocessing each of the spectra using a method as defined above; and training a discriminant model using the preprocessed spectra.

[0045] In another aspect there is provided an apparatus for optical biopsy, comprising apparatus for elastic scattering spectroscopy of tissue, including a light source for emitting light over a broad range of frequencies; a probe for transmitting light from the light source to tissue and for receiving light scattered in the tissue; a spectrometer for measuring the intensities of the received light as a function of frequency; and a processor for processing the measured light spectrum arranged to carry out the method as described above.

[0046] The apparatus may include a first optical fiber bringing light from the light source to a probe tip; and a second optical fiber bringing scattered light from the probe tip to the spectrometer; wherein the ends of the first and second fibers at the probe tip are arranged adjacently spaced apart by a predetermined distance.

[0047] The apparatus may include a decision processor for checking the fitted parameters against the results for one or more predetermined medical conditions and outputting the best fit medical condition based on the decision processor output.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] Specific embodiments of the invention will now be described, purely by way of example, with reference to the accompanying drawings in which:

[0049] FIG. 1 shows an optical biopsy system;

[0050] FIG. 2 is a flow diagram of a preprocessing method according to a first embodiment of the invention;

[0051] FIG. 3 is a flow diagram of an optical biopsy method using the preprocessing of FIG. 2;

[0052] FIG. 4 is a first example spectrum. taken from normal tissue;

[0053] FIG. 5 is an example spectrum showing interference from background illumination;

[0054] FIG. 6 is an example spectrum showing a small peak around 690 nm;

[0055] FIG. 7 illustrates normalisation;

[0056] FIG. 8 shows haemoglobin absorption; and

[0057] FIG. 9 shows the spectrum after haemoglobin absorption has been compensated.

DETAILED DESCRIPTION

[0058] The first step is to record an elastic scattering spectrum. Referring to FIG. 1, the apparatus for recording the spectrum includes an excitation light source 1 and a probe 3. A spectrometer 5 for splitting light and a detector array 7 for measuring the intensity of the split light are also provided. A first optical fibre 9 transmits light from the light source 1 to the end 11 of the probe and a second optical fibre 13 picks up light from the end of the probe and transmits it to the spectrometer for measurement. A computer 15 including interface electronics is electrically connected to the light source 1 the spectrometer 5 and the detector 7 for controlling these parts. The apparatus may be as described in U.S. Pat. No. 5,305,026 (discussed above) but this is not required and the skilled person will readily conceive of alternative probes, light sources and spectrometer arrangements.

[0059] In use, the end 11 of the probe is brought up to a tissue sample, such as the skin of a patient, so that the ends of the first and second optical fibre are adjacent to tissue. Light is then emitted from the excitation light source, passes through the first optical fiber 9 and into the tissue. After passing through and being scattered in the tissue some of the scattered light enters the second optical fiber 13 and passes to the spectrometer where the spectrum is measured.

[0060] In the specific example the excitation light source 1 is a xenon arc light which emits a number of pulses down the send fiber. The output detected at the spectrometer 5 is integrated to catch the response from the plurality of pulses. The spectrum is "auto-ranged" to ensure that the peak intensity at a chosen wavelength is scaled to lie above some threshold wavelength but below the saturation level of the detector 7, in this instance a CCD, by varying the number of light pulses. A second spectrum without illumination is obtained immediately before or after the illuminated spectrum is taken and the second spectrum is subtracted from the illuminated spectrum This removes effects due to extraneous light sources such as room lights, operating theatre lights or the headlamps of an endoscope.

[0061] The measured spectrum is then ratioed to a reference spectrum which is that obtained from a white material with constant reflection properties from the UV to the IR. Such a material is commercially available under the trade name "Spectralon". The resultant spectrum is then a ratio to a nominal mean intensity level 100. This removes any effects due to the spectrum of the light source. Next, the spectrum is smoothed using a simple "boxcar" function with a unit length of 7 pixels.

[0062] An example of the spectrum thus achieved is shown in FIG. 4. This is the spectrum that forms the starting point for preprocessing. The processing of the spectrum is carried out by a program 17 stored in the computer 15 for causing the computer to carry out the steps of the method that will now be described which will now be described with reference to FIG. 2.

[0063] The first step 102 is to define the useable wavelength range. This is principally determined by the noise in the spectrum and generally lies in the range 320-810 nm. Results outside this predetermined window are rejected. The skilled person will realise that when using different light sources to the Xenon light or different probe components useable results may be achieved over a different wavelength range and in that case a different wavelength window can be used.

[0064] The spectrum is then checked (Step 104) for measurement errors. In particular, it is common for the absorption peaks of oxygenated haemoglobin Hb0.sub.2 and deoxygenated haemoglobin Hb at 415 nm and 433%n to be saturated if too much blood is present in the tissue sample. That is to say, the measured light intensity may be substantially zero in this region. If this is the case the absorption is judged to be too great and the spectrum rejected.

[0065] The next check, in step 106, is to determine if the signal has been corrupted by background illumination. This can occur in one of two ways. If the illumination is too bright the CCD may become saturated. This can easily be checked for by determining if the peak signal level is too low; if it is the spectrum is rejected.

[0066] Another possibility is that sample movement between the tissue sample and the probe has occurred between the taking the illuminated and the dark measurements. This is a particular problem for endoscopic measurements made in the gut since gut tissue is highly active. This effect has been observed to give rise to a peak or trough between 600 and 655 nm. The existence of such a peak of trough is detected and if present the sample is rejected. Alternatively the recorded spectrum may be modified to eliminate this artefact.

[0067] A third check is then carried out in step 108 for good contact between probe and tissue. Imperfect contact appears to give a V-shaped feature at 690 nm, as illustrated in both spectra shown in FIG. 6, which can be checked for. Again, if the feature is present the spectrum can be rejected.

[0068] Assuming that the spectrum is acceptable after the above checks the next step in this example is to fit linear regions. It appears that the spectrum is approximately linear between 630 nm and 810 nm but better results are obtained by fitting in two regions, from 740 to 810 nm (step 110) and from 630 to 710 nm (step 114). There are a number of reasons for this. As mentioned below, blue dye can disturb the region below 730 nm. Secondly, the haemoglobin tail can extend to well above 630 nm. Thirdly, absorption due to water gives rise to a small non-linearity around 730 nm.

[0069] The fitting is carried out using conventional linear regression to fit a region of the spectrum to a straight line gradient m and intercept b. As is known, m and b are given by: 3 m = n i n x i y i i n x i i n y i n i n x i 2 - (i n x i) 2 b = i n y i i n x i 2 - i n x i i n x i y i n i n x i 2 - (i n x i) 2

[0070] where the subscripts i represent summation over the points in the fitted region.

[0071] Between fitting these two steps any absorption caused by blue dye may be removed in step 112. When used for the identification of sentinel nodes in breast tissue a blue dye, known as Patente Bleu V (Trade Mark) is used which has an absorption band with a peak at 635 nm. The blue dye spectrum is removed in the same way as used for fitting to haemoglobin features, as explained below.

[0072] After fitting to the 630 nm to 710 nm region the spectrum is normalised (step 116) to the gradient in the 630-710 nm linear section. In other words, the ratio to a line of constant gradient is taken for the whole spectrum so that the region of the spectrum between 630 and 710 nm becomes flat. This is illustrated in FIG. 7 which shows the original spectrum, a line of constant gradient fitted in the region between 630 nm and 710 nm and the normalised spectrum normalised so that the graph is flat in the region 630 nm to 710 nm.

[0073] Next, in step 118, haemoglobin lines are fitted and compensated.

[0074] In general, optical absorption is given by the well-known Beer-Lambert law, which states that the intensity of light remaining in a beam that has passed through a thickness z of material which has an absorption coefficient of .gamma. is given by I=I.sub.oe.sup.-z.gamma., where I.sub.o is the incident intensity. Conventional absorption spectra can readily be obtained in the published literature.

[0075] However, in tissue, the situation is more complicated and scattering effects must be taken into account. In elastic scattering spectroscopy, the situation is more complex still and the path-length through which photons travel through tissue is not well defined. Indeed, the path length varies non-linearly with the wavelength of light. Accordingly, published spectra of components may not be suitable and the absorption spectra used in the present invention are spectra measured using the equipment described above and in-vitro tissue phantoms. The spectra used thus implicitly compensate for the geometry and so the accuracy of absorption measurement and removal is enhanced.

[0076] For simple, one-component absorption, the amount of a component may be estimated using an offset from a straight line fit to an adjacent region. In the embodiment described, this is done by normalising the graph in the adjacent region and then calculating the offset from this absorption at the known frequency peak. This process is used for blue dye and beta carotene in the described embodiment, but as the skilled person will realise the process can also be used for other one-component systems.

[0077] For haemoglobin there is more than one absorber (Hb and HbO.sub.2) and the amount of haemoglobin must be measured using some form of fit. This is done by fitting the peak regions to a parabolic line shape and using a regression analysis in a known way.

[0078] Accordingly, the peaks in the absorption spectrum at which the measurements should be taken are defined. Referring to FIG. 8, which shows absorption spectra for oxygenated (HbO.sub.2) and deoxygenated haemoglobin (Hb), differences in the absorption profiles are obvious. After the normalisation of step 116, the wavelength positions and absorption coefficients in the vicinity of peaks in this spectrum are fitted to using a local parabolic approximation and multiple linear regression analysis.

[0079] It may be useful to find points of equal absorption. for both components and the absorption values for the second spectrum at positions corresponding to peaks in the first.

[0080] Once these values have been found as fit parameters these can be converted into absorption coefficients. The fitting values be used to calculate the saturation. defined as the percentage of oxygenated haemoglobin to total haemoglobin concentration, known as hematocrit.

[0081] The fitting parameters are then used to compensate the absorption represented by these fitting parameters and subtract that from the spectrum. The absorption calculated in the model from the fitted concentrations of haemoglobin is determined and subtracted (step 120) from the measured spectrum to obtain the spectrum used in subsequent steps. Two pairs of spectra before and after this subtraction are shown in FIG. 9. Spectrum 91 (before) becomes spectrum 93 (after), and spectrum 95 (before) becomes spectrum 97 (after) illustrated in FIG. 9.

[0082] The process of removal used in the embodiment relies on the assumption that the Beer-Lambert function describes the absorption sufficiently well and that the in-vitro absorption spectrum does not differ substantially to the in-vivo spectrum. The removal process is straightforward. The model used is simply to reverse the model used in the initial absorption process. Each point in the model spectrum is simply multiplied by a factor obtained from the fitted concentration by inverting Beer-Lambert's law to determine a model spectrum that can then be subtracted from the measured spectrum.

[0083] Although haemoglobin shows marked non-linearities in absorption at high concentrations this should not present a problem in samples that pass the test of step 104 above and have sufficiently little haemoglobin.

[0084] Although the subtraction does not, in general, completely eliminate the haemoglobin peaks, and indeed the effect is sometimes to invert the peaks, what the step does do is to remove from the spectrum those features already. recorded by the haemoglobin fitting parameters.

[0085] The skilled person will realise that there are a number of alternative ways to calculate the haemoglobin concentrations from the measured data. Rather than just fitting to the peaks, an alternative is to calculate the saturation from the isobectic point (the point at which both components are absorbed equally. The absolute absorption might then be used to calculate the total hematocrit.

[0086] In step 122, a linear fit is made in the region 540 to 630 nm.

[0087] In step 124, the absorption due to beta-carotene is fitted, recorded and then the absorption spectrum of beta-carotene subtracted from the measured spectrum. This fit takes place in a fitting range beginning at around 520 nm and extending down to include at least distinct peaks at 480 nm, 450 nm and 420 nm. Accordingly, the fitting range used may be all or part of the range 400 nm to 520 nm. Fitting to a Beta-carotene peak and consequently obtaining fitting parameters related to the concentration of Beta carotene is particularly important in pre-processing data from breast tissue, since beta carotene is related to vitamin C and found only in fat. Its presence is a contra-indication to malignancy.

[0088] Linear regions between 490 nm and 520 nm and between 455 nm and 480 nm are fitted. (step 126). As before, the gradients, intercept intensities and regression statistics are recorded.

[0089] Then in step 128, the residual absorption to other chromophoric components within the region of the Hb Soret band at around 415 nm is measured by parabolic approximation. This gives an indication of the different profile of cytokines and other tissue absorbers in the tissue as apposed to normal, whole blood.

[0090] Next, linear fits are provided in the regions of 340 to 360 nm and also 320 to 330 nm.

[0091] Although it may appear that a large number of fits have been carried out, the number of data points required to parameterise all of the fits is very much less than the number originally measured, which in the apparatus used is of order 1800 data points. The reduced number of data points can readily be used as the inputs to subsequent models, for example to train a model or to diagnose based on a previously trained model.

[0092] In order to use the data measured for diagnosis, it is first necessary to "train" a model by providing it with a number of fitted spectra for which it is known whether the tissue is normal or has a given medical condition in order to then be able to then use the test to determine whether another patient has that condition without the need for histology.

[0093] In the preferred embodiment, hierarchical cluster analysis is used. In hierarchical cluster analysis points in an n-dimensional space are grouped into a hierarchy of clusters. The grouping may occur from the bottom up, in which case each point is assigned to a single point cluster, and an algorithm groups pairs of clusters one after the other to produce a family tree

of clusters. Alternatively, it is also known to successively divide clusters to produce a hierarchy from the top down.

[0094] Hierarchical cluster analysis is known, for example for face recognition and other pattern recognition. Its use in diagnosis from elastic scattering spectra is mentioned in Paul M Ripley, D. Pickard et al. "A Comparison of Artificial Intelligence Techniques for Spectral Classification in the Diagnosis of Human Pathologies based upon Optical Biopsy" Novel Biomedical Optical Spectroscopy, Imaging and Diagnostics (Optical Society of America, Apr 200, Miami, Fla.) OSA Biomedical Topical Meetings Proceedings, 2000, MC5.

[0095] To train the model, a number of test samples of known diagnosis are taken (step 140); the samples are known as the training set. The training samples are then preprocessed as described above (step 142), and divided into clusters using hierarchical cluster analysis (step 144). Hierarchical cluster analysis is described, for example, in Duda, Hart and Stork "Pattern classification: 2.sup.nd edition", John Wiley & Sons, Calif., USA, 1998, and Andre Hardy "On the number of clusters", Computational Statistics & Data Analysis, volume 23 pages 83-96, 1996.

[0096] Each of the clusters is assigned a rating based on the points in the cluster. For example, if most of the points in the cluster based on the training set have the given medical condition, the cluster is labelled "suspicious". Conversely, if the majority of points in the cluster are clear of that condition, the cluster is labelled "clear".

[0097] Then, when an elastic scattering spectrum of a patient is taken, the point represented by the fitting parameters is determined and its Euclidean distance to each cluster is determined in the multidimensional parameter space spanned by the fitting parameters. If the point is further than a predetermined distance from any cluster, then a "don't know" result is given. Otherwise, the point is close to a cluster and it is assigned to the closest cluster and the label corresponding to that cluster is output as a diagnosis. This technique is known as the "leave one out" approach in hierarchical cluster analysis.

[0098] The method of the invention is particularly useful in diagnosing a number of conditions, for example breast cancer, dysplasia within the gastro-intestinal tract, cancers of the oral mucous, cervical cancer, lung cancer and skin cancer.

[0099] Although the invention has been described with reference to a specific example, the skilled person will realise that a number of variations are possible. In particular, a number of other predetermined components may be fitted for.

[0100] For example, a fit to the absorption line shape of bile pigment may be carried out in a fitting range including at least part of the range from 230 nm to 512 nm.

[0101] A fit to melanin absorption may be carried out using a large fraction of the whole spectrum as the fitting range; the fit may be carried out after fits to other absorbing components have been carried out and the absorption due to the other components subtracted from the measured spectrum.

[0102] A fit to protein absorption may be carried out below 350 nm.

[0103] Furthermore, although the invention as described above uses the wavelength as the abscissa of the spectrum, it is possible instead to use frequency or any other parameter related to wavelength as the abscissa.

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